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Metabolic Responses of Shallow Tropical Benthic Microcosm Communities to Perturbation

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FOREWORD

The Environmental Research Laboratory of the U. S. Environmental Protection Agency is located on the shore of Narragansett Bay, Rhode Island. In order to protect marine resources, the laboratory is charged with providing a scientifically sound basis for Agency decisions on the environmental safety of various uses of marine systems. This requires research on the tolerance of marine organisms and their life stages, as well as ecosystems, to many forms of pollution stress. In addition, a knowledge of pollutant transport and fate is needed.

The report that follows describes the use of flow-through aquaria for establishing, maintaining and monitoring shallow tropical benthic communities. Such studies are a logical intermediate point between laboratory bioassays and field surveys. The project is also intermediary between an earlier analysis of coral response to thermal stress and a present analysis of the responses of an entire ecosystem to the termination of sewage stress.

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Director
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ABSTRACT

Benthos communities simulating various aspects of coral reefs were established in 600-liter microcosm tanks. These communities were then subjected to various environmental perturbations, including altered light regime, altered substratum type, salinity depression, elevated nutrient level, and biological manipulation. The metabolic responses of the community to these perturbations were monitored, primarily by analysis of dissolved oxygen flux. Light, substratum type, and nutrient levels are resources which limit community metabolism. From 35 to 22 °/oo, metabolism is not sensitive to salinity. Salinities below 22 °/oo kill most test organisms. Metabolism is sensitive to biological manipulation.

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SECTION I

INTRODUCTION

Biological communities in natural settings are the product of a complex and largely unknown history of physical-chemical stimuli and organism responses and interactions. Attempts to explain the composition and function of communities and organisms in natural settings often do not arrive at a quantitatively satisfactory understanding of how the communities might change in response to perturbation. Biologists have frequently turned to laboratory or aquarium studies of single organisms or populations to avoid the complex and uncertain history of natural communities. Attempts to build a community analysis from such observations are ordinarily frustrated by the lack of realism in physical, chemical, and biological characteristics within the aquaria. The logical compromise is to maintain several species and trophic levels of organisms as an ecologically functional unit, and expose this unit to realistic and controllable physical-chemical conditions. The term "microcosm" has been applied to various artificially maintained biological assemblages that function as largely self-contained functional units. Beyers (1963) lists the following terms as having been applied to artificial aquatic ecosystems: microcosm, aquarium microcosm, carboy microcosm, microecosystem, experimental ecosystem, and laboratory-scale model. Other terms have appeared in the ecological literature since that time, including "synthetic microcosm" (Nixon, 1969), "gnotobiotic ecosystems" (Taub, 1969), and "artificial open systems" (Confer, 1972). The term "microecosystem," while perhaps more descriptive than "microcosm," is awkward and not entirely satisfactory. Thus, the term "microcosm" will be used throughout this report.

Using microcosms to study effects of environmental change in aquatic environments represents a logical advancement in the evolution of pollution research. Early aquatic pollution research was mainly concerned with such public health problems as the spread of disease through contamination of drinking water. During the 1930's and 1940's it became apparent that new tools were needed to understand the impact of pollution on aquatic systems. Efforts led to the development of the toxicity bioassay, which uses a single organism (usually a fish) to "model" the response of an ecosystem to pollution stress. During the 1950's and 1960's the toxicity bioassay became an increasingly sophisticated technique.

The earliest tests were short (24-hour) static tests. Further work showed that the toxicity bioassay model became more predictive of true biological impact as exposure time was increased (to 48 hours, 96 hours, and even longer) and if continuous flow was used. An extensive body of published information on proportional diluters and methods of analyzing mortality data developed. The model was refined by including considerations of synergistic effects such as natural light rhythm, temperature, oxygen tension, and

reproductive state; use of the most sensitive organism as an indicator further refined the assays. Another refinement was use of the most sensitive life stage of the organism as the bioassay model. The model was further refined by using measures of metabolism (e.g. fish respiration), rather than mortality, as the index of stress. Other advances led to the inclusion of a full range of test organisms (molluscs, crustaceans, etc.), in addition to fish, in the 14th edition of Standard Methods for the Examination of Water and Waste Water (Anonymous, 1976).

During the last decade, it has become increasingly evident that even more sophisticated and complex analytical techniques are needed to predict ecological effects of pollution. The study of complex multi-compartment living systems and their physical environments plays an increasing role in pollution research. It was inevitable that researchers would develop more complex simulations of complex natural systems. These simulations were microcosms, a logical extension from classical single-organism bioassays to a multi-component analysis. The microcosm includes all the features of the bioassay and differs only in complexity. The methodology, philosophy, and application of the bioassay that developed over the years led directly to microcosms. Use of metabolic variables, natural light regimes, prolonged sublethal stress, and continuous-flow apparatus originated with classical bioassay technique.

The microcosm provides an interface for interaction among various scientific disciplines. At the same time that pollution researchers were moving from single organisms towards organism interactions, ecologists were also in a period of transition. Classical ecology began with studies of natural systems in the field. Difficulties with interpreting and manipulating natural communities led to simplifications in the laboratory.

The microcosm is, at once, a complicated version of the pollution researchers' original bioassays and a simplification of the ecologists' traditional field observations. The microcosm approach will not replace existing techniques but will be used to study questions not amenable to present investigative methods. In order to include the microcosm technique in ongoing programs, one must consider potential uses and alternative techniques.

In many instances, the microcosm is clearly the method of choice. Population or community level trophic interactions are not predictable from data gathered on single organisms. Size and complexity of natural ecosystems ordinarily make *in situ* manipulation impractical. The microcosm represents an experimental approach which has been used successfully to isolate and analyze processes in aquatic ecosystems. Examples of this type of study include predator-prey interactions (Hall *et al.*, 1971), pelagic food-chain dynamics (Mullin and Evans, 1974), diurnal metabolic patterns (Beyers, 1963), patterns of autotrophic succession (Cooke, 1967), and the recycling of sewage effluent materials through marine food chains (Ryther *et al.*, 1972).

Microcosms may be constructed and scaled according to differing criteria. One criterion might be to attempt to simulate an entire ecosystem in terms of the concentration and relative abundance of major abiotic and biotic

components. Our criterion has been to simulate relatively restricted communities, or parts thereof. Ecosystem-level simulations can be achieved by linking (e.g. via water flow) community-level microcosms.

Microcosms represent the logical union of laboratory and field investigative technique. Hence, microcosm experiments utilize various types of laboratory techniques and concepts developed for use on single species (e.g. mortality measures, metabolic techniques, growth measures, etc.), combined with standard field sampling techniques and concepts (e.g. fouling panels, subsampling the community for epifauna, infauna, plankton and other components, sediment analysis, community photosynthesis and respiration, etc.).

The various techniques of microcosm analysis fall into two broad categories: functional analysis and structural analysis. Odum (1962) defines biological "structure" of an ecosystem to be the composition of the biological community including species, numbers, biomass, life history, and distribution in space of populations. That is, biological structure is the nature of the biota in an ecosystem at an instant in time. Odum defines "function" to be the rates of material, energy, and information flow through the ecosystem.

Function is often amenable to direct total-system monitoring; such integrative monitoring reduces the need for the laborious and inherently imprecise summation of structural components to understand the system. For example, many organisms contribute to the biomass of a community; carbon flux, which is a time-differential function of biomass, can be directly measured in the water column and can be used to assess the change in biomass with time. The assessment is properly weighted and integrated according to the contribution of each component to ecosystem changes, whereas biomass need not have any direct and/or constant relationship to energy flow or material flux. Moreover, non-destructive methods of measurement can be employed with functional assessment.

Because microcosm technique invites the simultaneous use of numerous methods and involves very complex multi-compartment systems, it is vital not to lose sight of this unifying relationship between structure and function. Ultimately the goal of environmental research is to predict the exact nature of the structural/functional relationship for ecosystems influenced by human activity.

Inclusion of microcosm facilities in a well-planned environmental program provides many research advantages, but the technique must also be evaluated in terms of time and cost. Advantages and disadvantages which will ultimately influence utilization of continuous-flow microcosms can be summarized as follows:

Advantages

1. Microcosms are the most suitable method available for studying certain community-level processes; the size and complexity of natural ecosystems make them impractical to manipulate experimentally. Experiments based on single organisms do not predict community responses. The microcosm

consists of major biological components of natural communities, and therefore should be a better tool for predicting ecosystem responses to perturbation than are single organisms.

2. Various structural and functional responses can be observed simultaneously. Growth, reproductive state, recruitment, mortality, individual component metabolism, material and energy pathways can be identified and measured. Sensitive indicator species for a given stress can be quickly identified.

3. Microcosms are extensions of the natural world. Normal diel variations in ambient conditions can be duplicated. Natural foods and recruitment are provided. Normal seasonal variations are followed, and natural seral development is allowed.

4. Wastes, biogenic toxins, hormones, and pheromones can be kept at near-natural concentrations.

5. Microcosms represent a middle stage between controlled laboratory experiments and *in situ* field observations.

6. Microcosms offer research flexibility. Units can be connected in series; thus progressive de-toxification or stripping processes may be studied. Conversely, a single unit may be simultaneously used for a number of non-destructive studies. Microcosms may be used as a sorting technique for the identification of potential bio-indicators.

7. Various perturbants can be introduced into replicate microcosm communities or communities with specified differences under controlled conditions. Sub-lethal stress levels may be used, because long-term experiments are feasible; such experiments also lend themselves to chain-response studies in complex communities.

8. Electrochemical probes and automatic recording systems can be added to the microcosms, facilitating continuous observation. Large amounts of data gathered in this manner can be processed automatically.

9. Because microcosms can be more precisely controlled and monitored than the natural systems they simulate, the microcosms are amenable to the validation of mathematical ecosystem models. Accurate mathematical descriptions of natural systems are a major objective of the environmental sciences, because only with high-speed simulation of ecological pressures can a proper evaluation be made of the likely long-term environmental effects of management decisions.

Disadvantages

1. The cost of a complex microcosm facility is high, and considerable lead time is required to build such a facility.

2. Nearly continuous pump operation is required in flow-through microcosms; there are high maintenance costs, as well as considerable experimental risk of breakdown.

3. Biological recruitment into the system may be largely uncontrolled, or controlled with difficulty.

4. There are constraints on the manipulation of certain variables (e.g. salinities higher than ambient are possible but difficult) and also on the size of the microcosm. Although size limits have not been thoroughly investigated, not all components of a given community (large carnivores, for example) can be effectively accommodated.

Within this context of potential and realized utility of aquatic microcosms, this report describes the design and utilization of microcosms appropriate for shallow tropical benthos communities. Because these communities may be complex, heterogeneous, fragile, and metabolically active (e.g. coral reefs), we have been forced to consider and overcome numerous specific operational problems. Many design characteristics of the resultant product, as well as many of the scientific findings, can be transferred to other situations. We refer, in particular, to the report by Henderson *et al.* (1976) comparing the Hawaii Institute of Marine Biology microcosm facility with a companion facility designed by the Naval Undersea Center.

The focus of this study has been the design and construction of an experimental microcosm facility for the culture of integral benthic communities, under conditions which are more representative of natural situations than simple laboratory aquaria, and at the same time, are easier to control, manipulate, and monitor than the complex communities of real-world coral reefs.

Design, construction, and utilization of the facility were interrupted by funding breaks between each fiscal year of the program. These interruptions complicated the maintenance of personnel, continuation of experiments, and execution of a well-designed experimental chronology. As a result, the specific environmental lessons learned from our efforts are less than they might have been. Nevertheless, we have extracted a series of specific accomplishments which demonstrate the utility of the microcosm facility and provide insight into shallow tropical benthos ecosystems.

There have been numerous spinoffs of this program. CO_2 has been investigated as a metabolically useful variable, primarily in situations other than the microcosms. We have participated with other groups in field studies at Canton, Christmas, Enewetak, and Fanning Atolls, in the tropical Pacific Ocean. We have worked with the Naval Undersea Center in the design and utilization of a second microcosm facility.

SECTION II

CONCLUSIONS

1. General conceptual designs are presented for a microcosm facility appropriate for simulating shallow tropical benthos communities. These designs present major considerations for the effective operation of such a facility.

2. The microcosm facility proves to be a useful tool for the study of benthic communities over periods of several months. The fouling communities which develop in the microcosms can be complex, and organisms deliberately introduced into the facility function normally.

3. Microcosm tanks built according to our design can be characterized by a physical flushing model of total mixing; for these microcosms, O_2 production has proven to be a useful metabolic measure. Gas exchange can be ignored.

4. Shallow benthos communities which have been set up in the tanks are demonstrably light-limited. The solar conversion efficiency of these communities is high, so naturally or artificially turbid water is likely to be a significant detriment to the rapid productivity characteristic of reef communities.

5. Substratum is a second resource which limits the metabolic activity of microcosm communities. Substratum type is often altered by artificial activity.

6. The microcosm communities are also limited by nutrient availability, even though the nutrient loading at our primary microcosm site is high. Again, nutrient loading is a variable often altered by human impingement on reef ecosystems.

7. At least three variables (light, substratum, nutrients) are resources which may simultaneously limit reef production. Thus, heterogeneous benthos communities, even as simplified in the microcosm situation, do not conform to Liebig's Law of the Minimum.

8. Salinity down to 22 ^{o}/oo does not have a demonstrably deleterious effect on reef ecosystems. The damage done to reefs by stream runoff must therefore be attributed either to lethal effects due to extreme salinity depression or to associated variables such as siltation or toxicants.

9. Within broad limits, organic carbon production by benthic communities with varying community structure is similar. If grazing pressure is altered, metabolic response is responsive to quantity (but not particularly to quality) of altered grazing pressure.

SECTION III

RECOMMENDATIONS

1. Microcosms should be established and used as logical extensions of standard bioassay techniques in order to gain a more adequate understanding of how ecosystems respond to stress.

2. The results reported here are restricted to community oxygen metabolism. Examination of CO_2 and nutrient fluxes should be undertaken, although oxygen metabolism is by far the easiest variable to automate. Community structure in the microcosms can also be examined, although variables related to structure are almost as "noisy" in the microcosms as in natural situations.

3. The oxygen metabolism data are examined by analysis of covariance, with the regression analysis being a convenient method to screen out the primary community response to variable (and largely uncontrolled) light levels.

4. Extended, systematic experiments should be conducted to validate and refine the results of preliminary experiments reported in this document. Light, substratum, and nutrients are all demonstrably important to reef metabolism and should be investigated further. Salinity effects appear surprisingly minor; this point should also be examined in more detail. Biological manipulation is also an important control of community metabolism. All of these variables are of practical importance to environmental protection /alteration. Numerous other variables (temperature, toxicants, water motion, etc.), should be investigated as well.

5. As reported here, perturbations were applied as relatively constant "stresses." Further experiments should be performed to examine not only the intensity of perturbation but also the duration and frequency of perturbation. Our preliminary analyses suggest that the first-order stresses are of more immediate concern than synergistic perturbations, although synergisms should eventually be examined as well.

SECTION IV

UTILIZATION

A. Description of Microcosms Used in this Study

A detailed description involving the design, construction and operation of the HIMB-NUC microcosm facilities is in the Appendix. The following section describes briefly specific experimental aspects of the microcosm units.

The microcosm tanks are 117 cm square by 46 cm deep fiberglass containers (Figure 1). A continuous gravity-regulated jet (normally 10 liter min^{-1}) of seawater enters the microcosm through a port in the center of the floor and leaves the system through a drain in the floor corner. This arrangement provides mixing of the water without excessive agitation, local turbulence or bubble formation. Violent entrapment of air alters dissolved oxygen flux used to calculate metabolism, so such air entrapment must be avoided if community metabolism is to be measured. Mixing characteristics of the microcosms are discussed elsewhere in this section. The microcosm tank is a well-mixed, continuous-flow, 630-liter reaction vessel with a mean water residence time of approximately 1 hour. Inlet and outlet water composition can be sampled automatically for some variables (dissolved oxygen, pH, temperature) or manually for others (inorganic nutrients, particulate load, plankton, alkalinity, etc.).

Alteration of chemical, physical, or biological characteristics of the influent water is carried out before it enters the microcosms. The particular tanks used in this study are a manageable size suitable for simulating the community structure of shallow tropical benthic systems.

B. Manipulation of the Microcosm Environment

The effort to design adequate microcosm facilities was directed at producing various living multi-compartment representations of reef communities, and the means of manipulating the environment within these communities. During this development program, a variety of factors have been manipulated:

1. Chemical factors--The initial manipulation of water chemistry was aimed at dissolved nutrient elevation. During the studies reported here, nutrient elevation was accomplished by mixing relatively low-nutrient water from Kaneohe Bay with high-nutrient water from a seawater well. Subsequent nutrient-loading experiments have been accomplished by using a precision peristaltic pump to add concentrated nutrient salt solutions at 1 to 100 ml min^{-1} to the predominantly low-nutrient flow (10 liters min^{-1}). Copper

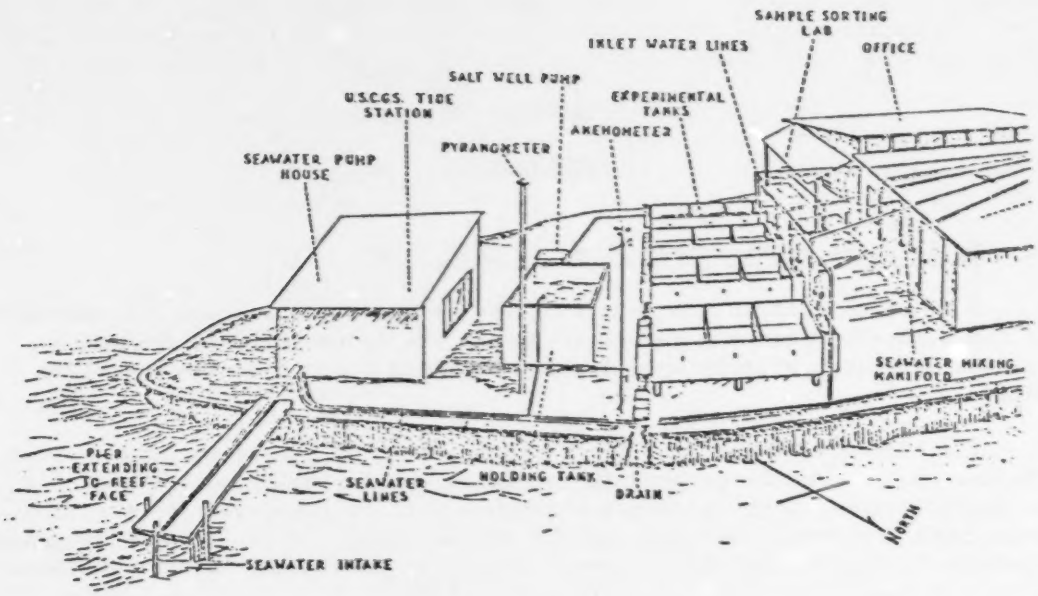

Figure 1. Configuration of the HIMB microcosm facility.

additions at the NUC facility have similarly been effected by the addition of concentrated salt solution. In both the reagent additions and the bay plus well mixtures, the microcosm head boxes serve as mixing chambers.

Salinity alteration was accomplished by adding municipal fresh water from a large (10 m^3) holding tank maintained at a constant head by a vertical standpipe and an excess of freshwater input. Water was gravity-fed at a constant flow rate from this tank to the microcosm inlet boxes. This procedure prevented possible variation in delivery rate from variation in line pressure.

Dissolved oxygen levels were lowered by bubbling nitrogen through the seawater headbox. The degree of O_2 lowering may be adjusted by varying the rate at which the N_2 is bubbled through the inlet head box.

2. Physical factors--Light is one of the most important physical variables, because it represents the major energy source for plant communities. The light intensity, spectral quality, and photoperiod encountered on tropical reefs cannot be easily duplicated in a laboratory. Fortunately, there is

no reason to duplicate such conditions, because microcosms can usually be located outdoors in full natural radiation. Reduction of light below ambient levels (such as might occur with high water turbidity) can be simulated by placing various layers of different neutral-density screening over the microcosms to reduce light. By use of multiple screens of various densities, we have worked at light levels between 2 and 70 percent of full sunlight.

Temperature is altered by heater-chiller units as described by Jokiel *et al.* (manuscript). Substrata of various types are added as required. Mud, sand, rubble, and hard substrata have been utilized in this program. Water motion is increased by positioning motor driven agitators of various horsepower on the microcosms.

C. Methods of Structural Manipulation and Analysis in Microcosms

The microcosm communities used in this study are complex in biological structure. Several hundred species of organisms commonly establish themselves, entering the microcosms as larvae in the seawater. These communities are rich, complex, and responsive to changes in microcosm environment (e.g. alteration of substrata, water motion, nutrient loading, light regime, presence of large grazing fish, etc.). As in the case of natural communities, an orderly community succession occurs (Table 1). An initial bloom of algae is followed by settlement of rapidly growing herbivores such as the sea hare *Stylocheilus longicauda*, which crop back the algae. Other organisms settle; in time (usually several months) carnivores and omnivores settle, and a community which will remain relatively stable through time develops.

Transplanting intact communities from the field can shorten the period or alter the characteristics of succession and growth. Transplanted communities are less dependent than the fouling communities on larval recruitment, and stabilize to ambient tank conditions rapidly. Substrata with associated organisms are removed from the field and transported to microcosms. The microcosm community can also be structured by adding fishes, corals, or other fully grown organisms that otherwise would enter randomly and grow towards adult size during the course of the experiments. Some large benthic organisms of a coral reef have lifespans of months to years, and community succession in reefs is both complex and slow. It is therefore impractical to wait for larval recruitment to produce a truly mature community in the microcosms.

As experiments are conducted, routine structural subsampling can be carried out, usually using many of the same techniques that are employed in field programs. Various subsampling routines have been employed, including subsampling the substrata, scraping small sections of the wall, or utilizing fouling panels. Photography is a satisfactory record for many analyses. Various methods of statistical analysis of community structure may be applied. Fishes, coral, and other large biota can be removed from the tanks, weighed individually, and then returned to the tanks.

D. Methods of Functional Analysis in Microcosms

The results presented in this report deal primarily with community function of the microcosms. Measurement of microcosm community function

TABLE 1. AN EXAMPLE OF TYPICAL COMMUNITY SUCCESSION

Chronology of first appearance of dominant biota in microcosms at the NUC facility (Spring 1975), starting with "sterile" microcosms (from Henderson *et al.*, 1976).

Weeks of Flow-Through	*Algae*	*Cnidarians*	*Crustaceans*	*Annelids*	*Molluscs*	*Echinoderms*
1+	diatoms cyanophytes filaments and tufts of algae					
2+						
3+	green tufts				*Stylocheilus longicauda*	
4+			amphipods			
5+	calcareous algae				*Cerithium nesioticum*	
		Aiptasia pulchella			bubble shells	
6+	brownish algae tufts				*Cheilidonura hirundindina*	Synaptidae sp.
					other nudibranchs	
7+	*Ectocarpus* *Valonia* *Laurencia*				*Aplysia parvula* *Aplysia juliana*	
8+			portunids			
9+					*Dolabrifera dolabrifera*	
10+			*Percnon planissimum*			
11+					*Dolabella auricularia*	
					vermetids	
12+				*Hydroides* sp.		
			alpheids	spirorbids		
	Padina					
13+	*Hydroclathrus* *Colpomenia*					
14+		*Hippolysmata kukenthali*			*Strombus maculatus* *Cerithium sinensis*	
15+		*Gnathophyllum faciolatum*				
					Cypraea caputserpentis	
16+						
17+						*Echinothrix* sp.
						Stichopus horrens
18+						
					Pinna muricata	
19+						

involves the measurement of net flux of various biologically active materials (e.g. oxygen, carbon dioxide, nitrogen compounds, and phosphate) through the system. Oxygen is used most often because it is easily monitored, can be recorded continuously with a polaragraphic cell, and is a direct measure of

net photosynthesis and respiration. There is ordinarily relatively little variation in the inlet oxygen concentration, so the flux calculations are straightforward (see discussion below). Such measurements are by no means restricted to oxygen.

Flux of nitrogen or phosphorus is determined by measuring outlet and inlet concentrations of the various biologically active forms of these elements. Large variations in the inlet nutrient levels make nutrient fluxes difficult to calculate without large numbers of analyses. Measurement of total carbon flux can be determined using pH, alkalinity, dissolved organic carbon and particulate organic carbon measurements. Net calcification within the community can be determined from alkalinity changes alone. Smith and Key (1975) summarize the use of CO_2 as a metabolic record in marine ecosystems. Flux of toxicants such as introduced heavy metals, or chlorinated hydrocarbons, as well as their effect on community metabolism, can be measured. After a period of addition, rate of de-toxification and recovery can be similarly determined.

If inlet concentration, outlet concentration, and flow rate are known, the total flux of the material for a steady state microcosm would be calculated as (outlet concentration - inlet concentration) times the flow rate. Steady state conditions are not usually obtained, because most metabolic processes follow a diurnal rhythm, and because of short-term variation. Hence, mixing characteristics of the microcosm must also be considered.

E. Flushing Characteristics of Microcosms and Natural Ecosystems

In order to measure community metabolism, one must understand the flushing characteristics of the ecosystems in order to apply appropriate calculations to the conservative (advective or diffusive) and nonconservative (*in situ* uptake or release) fluxes of metabolically relevant variables. One may imagine three end members of flushing models on a triangular diagram: non-mixing stream flow, complete-mixing with continual exchange, and pulsed exchange. Real world conditions are ordinarily intermediate among these end members, but many practical situations cluster near one of these conceptual extremes. It is often possible to take advantage of a natural or artificial tracer which is strictly conservative in order to describe the appropriate flushing model. Smith and Pesret (1974) and Smith and Jokiel (1976) have applied such a technique with salinity in coral reef ecosystems (see also Smith, 1974).

It would appear that the complete-mixing, continual-exchange model most appropriately describes the characteristics of the microcosms. If this hypothesis is correct, then any instantaneous alteration of a totally conservative property of incoming waters should be characterized by an exponential decay curve from the pre-alteration state to the post-alteration state. The decay constant should be the inverse residence time of water in the tank (i.e. the flow rate of water through the tank divided by tank volume).

Salinity changes during a salinity-alteration experiment demonstrate the validity of this complete-mixing model for our tanks. The salinity of the inlet box was abruptly dropped 10 °/oo below ambient (from about 35 °/oo to 25 °/oo) by an admixture of fresh water, and the salinity in the tanks was monitored for four hours. Figure 2 demonstrates that, after an initial lag (related, perhaps, to density stratification) the salinity drop was well approximated by a negative exponential (mixing) curve. The exponential slopes yielded water residence times ranging from 53 to 64 minutes; these residence times agree well with the 60 minute residence time being approximated during that experiment by the tank flow rate.

The general validity of the complete-mixing model for the microcosms has been established with conservative properties. We can now turn to nonconservative properties and superimpose these on the model. For a known residence time, we can now develop the appropriate equations. Let the subscripts 0 and t denote samples at times 0 and t minutes later; τ is the residence time of water in the tank. X is the inlet concentration and Y is the outlet concentration; "a" is a subscript denoting the concentration change of Y attributable to advective flux; "r" denotes the concentration change attributable to internal reactions. V is the tank volume; Z is the tank depth. F is the flow rate of water through the tank.

The residence time may be calculated:

$$\tau = V/F \tag{1}$$

The advective concentration change at time t may be approximated by:

$$Y_a = [(X_0 + X_t)/2 - Y_0] \cdot [1 - e^{-t/\tau}] \tag{2}$$

$(X_0 + X_t)/2$ is an estimate of the mean inlet concentration over the interval in question. The reactive concentration change at time t is:

$$Y_r = Y_t - Y_0 - Y_a \tag{3}$$

Y_a and Y_r may be reduced to rates of advection and reaction per unit area by dividing by the time increment and multiplying by water depth.

Such calculations form the basis for our estimates of advective flux and metabolic flux in the microcosms. The method and the rationale behind it have been described by Northby (1976).

The microcosms can also be used according to a pulsed exchange model, simply by stopping the flow through the tanks, removing some volume of water, and replacing that volume by new water. We have used this latter configuration under particular situations, and such pulsed exchange indeed does characterize some tidepools or large isolated water bodies in particular coral-reef settings (e.g. Kinsey and Domm, 1974).

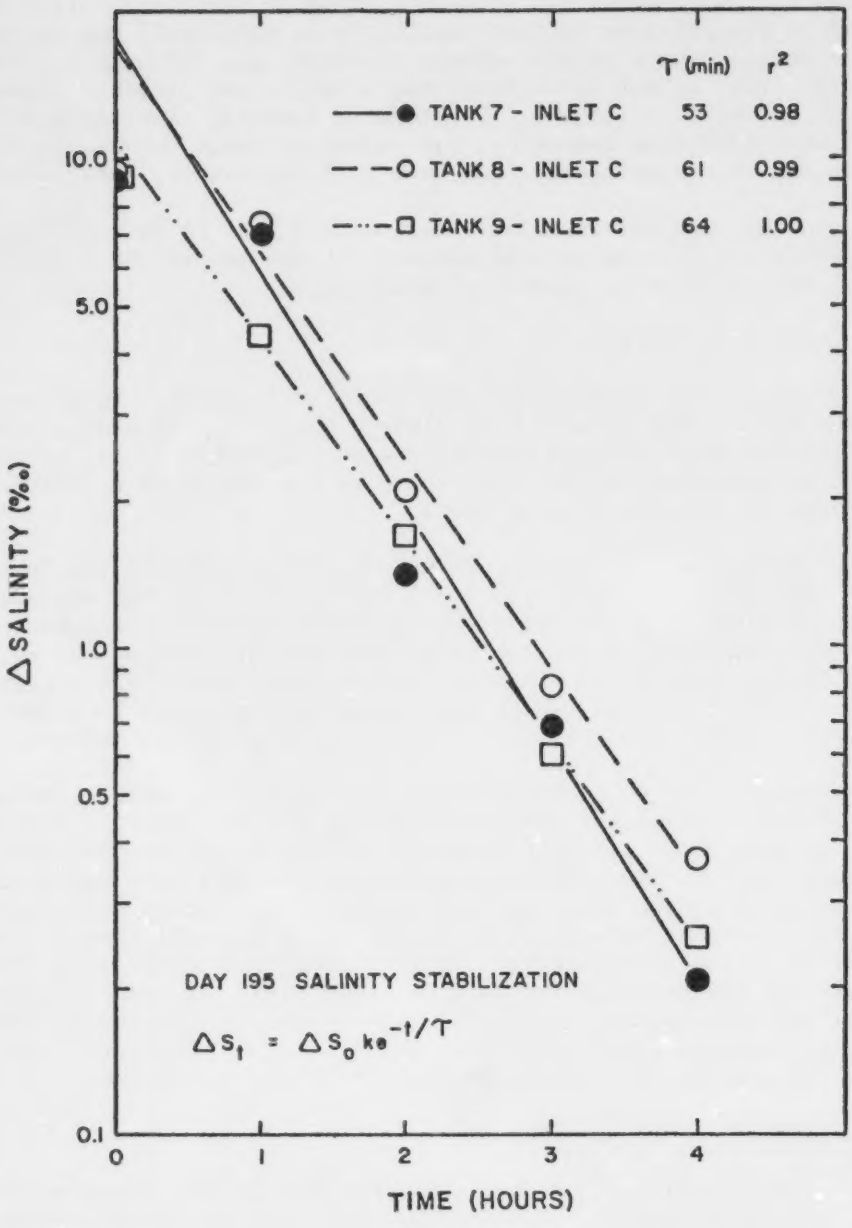

Figure 2. Return of altered salinity to ambient as a function of tank flushing.

F. Gas Exchange in HIMB Microcosms

Measurements of metabolic rates according to the general flux model developed in the previous section implicitly or explicitly assume that advection of water and state changes within the water are the only two processes of concern. That is not quite true; there can be gas exchange of materials across the air-water interface. Oxygen is a commonly (including in our studies) used metabolic indicator, and oxygen exchange across the air-water interface can be a significant flux term (e.g. Kanwisher, 1963; Kinsey, 1973).

In general, gas exchange rate (R, moles m^{-2} hr^{-1}) is a simple function of the difference between partial pressure of the gas in the atmosphere (P_g, atm) and the aqueous partial pressure (P_w):

$$R = K \cdot (P_g - P_w) \qquad (4)$$

K is an exchange rate coefficient (moles m^{-2} hr^{-1} atm^{-1}). In an open-water system subject to wind stress, K is clearly a function of wind stress. The explanation for this relation between K and wind speed (V) is, according to the model of Kanwisher (1963) that there is a boundary of a finite thickness which varies in response to speed.

We have measured O_2 exchange in our microcosm tanks in the following manner. Flow through a tank with no contained organisms was stopped. The upwelling of water from the inlet was simulated by putting a submersible bilge pump, outlet up, in the center of the tank and its pumping rate was adjusted to approximate the ordinary rate of water flow. The oxygen level of water in the tank was elevated by bubbling with compressed gas; then that bubbling was stopped and the loss of O_2 from the water was measured.

The measured oxygen exchange rate constant for the microcosms under a variety of wind conditions averaged 43 mmoles m^{-2} hr^{-1} atm^{-1}. This rate is near the no-wind value given by Kanwisher (1963) and Kinsey and Domm (1974). We conclude that the rim around the margin of the microcosm tanks and the negligible fetch in the tanks combine to reduce gas exchange to a minimum. The observed exchange rate constant is sufficiently small that we can ignore this process in our calculations. It is generally accepted (e.g. Kanwisher, 1963) that CO_2 gas exchange is small relative to O_2, at least in part because generally less than 1 percent of the CO_2 in seawater is present as free CO_2. The nominal exchange rate coefficient of 25 moles m^{-2} hr^{-1} atm^{-1} can be used for CO_2; the process can, again, be ignored in the microcosms.

G. Relationship of ΔO_2 to ΔCO_2

Most older studies of coral-reef metabolism relied on measurements of O_2 changes, assumed a metabolic quotient of -1.0, and calculated carbon flux directly from O_2 data (either with or without a gas exchange correction). Qasim and Sankaranarayanan (1970) did not assume that $O_2/\ CO_2 = 1.0$, but rather used Ryther's (1956) suggested "best value" of -1.2. Smith and Marsh (1973) examined O_2 and CO_2 data for Enewetak Atoll and concluded that the

best general relationship between O_2 and CO_2 is indeed very near 1. They noted that there was apparently a more scattered data set in the night samples, and they attributed this to problems in resolving CO_2 changes at night.

We have measured O_2 and CO_2 in microcosm runs in which the dominant organisms were a fouling diatom community. These data again yielded O_2/ CO_2 values very near -1.0, with greater scatter in the nighttime. For the most part, O_2 is an easier measurement to automate than is CO_2; therefore we report O_2 here and assume that the appropriate metabolic quotient is near -1. This is almost certainly an oversimplification. The quotient does vary, and it is likely to be a useful environmental indicator in its own right. We will not pursue that point further in this report.

Carbon dioxide changes in seawater can be related to another important metabolic process associated with coral-reef systems: calcification. Although we do not pursue CO_2 analyses in this report, considerable effort has been devoted during this investigation to the development of appropriate analytical procedures for measuring metabolic processes via the CO_2 system. A publication derived from this study (Smith and Key, 1975), describes the utility of the CO_2 system as a metabolic record in calcifying ecosystems, while another paper (Smith and Kinsey, 1978), describes the methodology for CO_2 measurements in seawater. Under most conditions simulated in the studies reported here, calcification has been very slow. Subsequent investigations (Jokiel, 1978) have demonstrated that coral calcification is sensitive to water motion, a variable which ordinarily assures low values in the microsms.

H. Replication and Reliability of Metabolic Measurements

In essence, the utility of any environmental variable as a legitimate and quantifiable measurement of environmental status can be reduced to a single question: Can the particular variable be easily used to determine statistically reliable differences between environments in order to provide a practical means of environmental assessment? Some environmentally valuable variables are sufficiently slow or difficult to measure that they fail the second half of this question; unfortunately, many promising measurements of biological community structure fall into this category. Other easily measured variables (including many of the chemical parameters which have legal status) fail to have demonstrable environmental utility within statistical limits. In general, the problem with using such variables lies with the lack of experimental strategy associated with their measurement rather than with lack of inherent utility of the variables.

Where they can be used, metabolically active chemical variables may be relatively easy to measure. In at least some environments, these variables also appear to have environmental significance. In the following section, we will examine patterns of metabolic variation within versus between sample treatments. The data presented are a series of O_2 metabolic rates obtained for microcosm communities.

We extend conclusions about the utility of oxygen metabolism in the microcosms to O_2 metabolism in many real world situations. In those situations, gas exchange may override the metabolic effects of O_2metabolism, so CO_2 (including $CaCO_3$), inorganic nutrient, and other metabolic fluxes are likely to prove more useful than O_2. Data from the lagoon of Canton Atoll (Smith and Jokiel, 1976) provide a case of point. In that study, we related budgets of carbon, nitrogen, and phosphorus to physical and biological gradients. We suggest, based on the analyses presented in this report and on the Smith/Jokiel studies, that careful mass-balance budgets of many metabolically active materials are probably of more use as environmental measurements than are the absolute values of these components. Unless the components in question are likely to be major environmental stimuli or depressants as they deviate slightly from the norm, their rates of deviation are likely to be more significant than their absolute deviation.

SECTION V.

MICROCOSM COMMUNITY METABOLISM

The purpose of the studies reported in this section is to examine the responses of microcosm community metabolism to changes in environmental conditions. The experiments reported here use biological oxygen flux as the primary community metabolic measure and evaluate the metabolic changes that result from the following forcing functions: light, substratum, nutrient levels, salinity, and direct manipulations of community structure. The results are by no means final and are not intended to establish legal or environmental guidelines. Rather, they are intended to develop and demonstrate analytical technique for microcosm studies.

After examining several approaches to the data analysis, we have concluded that it is most useful to present the results in terms of one-way or first-order effects. Some experiments were conducted in a matrix format to look at synergistic (or cross-product, or second-order) treatment effects. Invariably the first-order effects dominated, and higher order effects were barely (or not at all) detectable above the error terms. We have therefore chosen to present the results as a series of single-order experiments. It is obvious that more detailed and exhaustive analyses of community metabolism responses to environmental variability should be undertaken to define the higher order effects which surely exist, and these analyses should also examine duration and frequency of perturbations as well as quality and intensity of perturbations. Those examinations proved to be beyond the practical scope of these studies, partly due to time losses from funding interruptions.

A. Light

Shallow benthos communities in tropical environments (as represented by our microcosms) are ordinarily dominated by plants, so a forcing function which exerts major influence on the metabolism of these communities is light. The absolute light level has not been controlled (other than "off" or "on") in the outdoor microcosms during the course of the experiments reported here. Neutral density filters have been used in other experiments to vary the relative intensity between treatments. Figure 3 is a typical curve of oxygen metabolism and light level versus time of day. Two of the tanks (T1 and T3) represent variously manipulated fouling communities developed in tank microcosms over a period of 38 days. The third tank (T12) shows the calculated flux associated with water flowing into a clean tank; this may be interpreted as an evaluation of the "ambient water blank" plus the analytical error of the method.

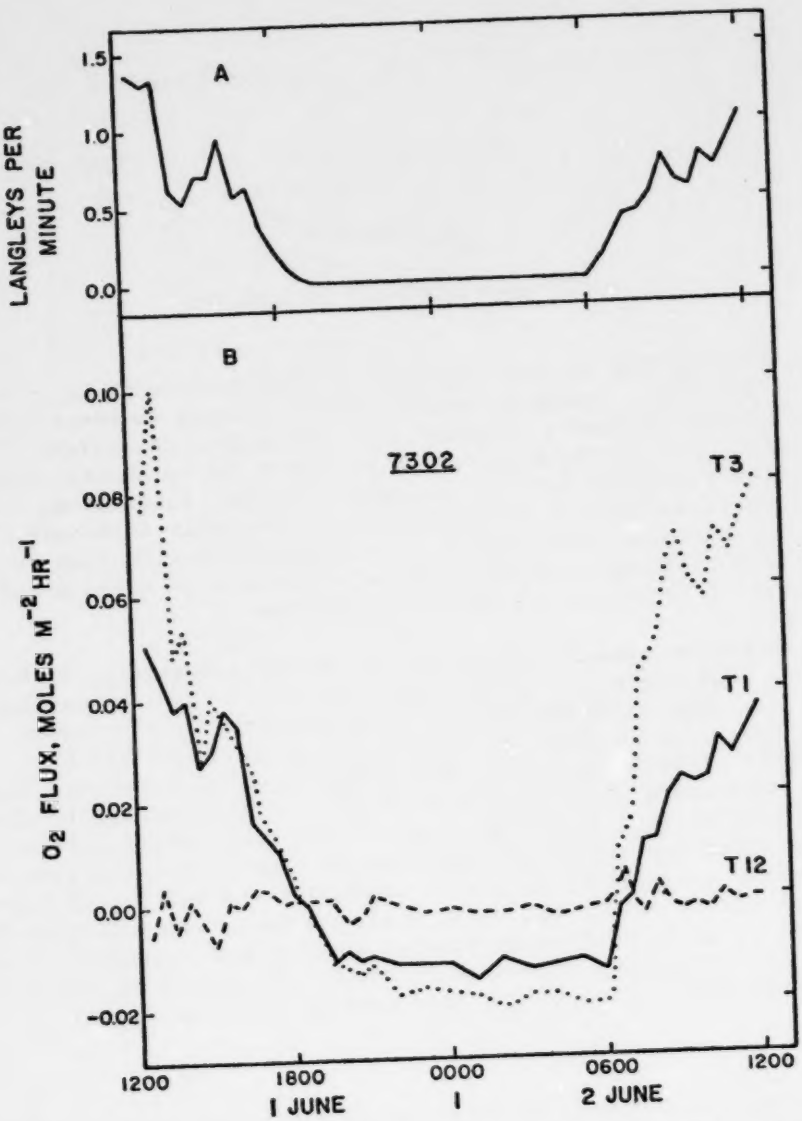

Figure 3. A. Curve of ambient solar radiation over 24 hours. B. Diel response of oxygen flux in two tanks with fouling communities (T1, T3) and one tank with no significant biotic component (T12).

Clearly, responses to light dominate metabolism in these microcosm communities. It is, therefore, necessary first to calculate the metabolic response to light and then to examine either residuals within a particular photic response pattern or differences between responses.

Figure 4 presents four scatter diagrams of half-hour increments of solar radiation versus oxygen metabolism. The community in question was a

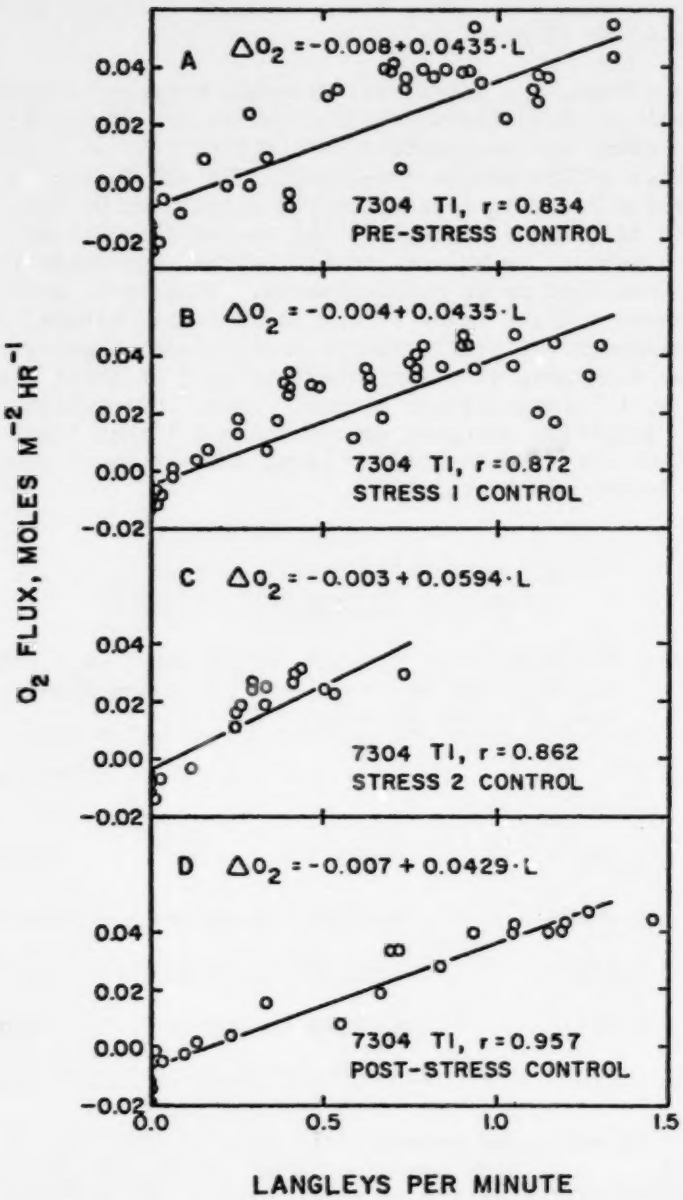

Figure 4. Solar radiation versus oxygen flux for four successive measurement periods in the same tank.

diatom-dominated fouling community which developed on rubble substratum and tank walls over approximately one month previous to the first metabolism measurements. Water began flowing in the tanks on 9 June 1973, and diagrams A through D represent data collected between 10 July and 31 July. Several observations can be offered.

For this series, the linear relationship between light and oxygen metabolism is excellent (correlation coefficient varies from 0.83 to 0.96). Nevertheless, there are suggestions that the functional relation between light and oxygen metabolism is non-linear. In all instances, the y-intercept of the regression lines overestimates the calculated 0-light oxygen flux (Table 2). In diagrams A through C, the oxygen flux values at intermediate light levels appear to be underestimated by the regression lines while the high-light values tend to be overestimated. Diagram C, which has only two data points above a light value of 0.5 langleys per minute, appears to have a slightly (although not statistically significant) steeper regression slope than the other diagrams, with approximately half of their light values between 0.5 and 1.5 langleys per minute. These observations are all consistent with the generally accepted conceptual model that light becomes progressively less limiting to community or plant metabolism at progressively higher levels (e.g. Jassby and Platt, 1976).

TABLE 2. REGRESSION EQUATIONS (in the form $\Delta O_2 = a + b \cdot Light$) FOR DATA PRESENTED IN FIGURE 4

Parameters ± 1 standard error unit. For comparison the directly observed 0-light oxygen fluxes ± 1 s.e. are also reported

	b ± s.e.	a ± s.e.	0-light O_2 flux ± s.e.
A	0.0435 ± 0.0051	-0.00813 ± 0.00384	-0.01775 ± 0.00189
B	0.0435 ± 0.0036	-0.00356 ± 0.00228	-0.01050 ± 0.00078
C	0.0594 ± 0.0084	-0.00338 ± 0.00292	-0.00860 ± 0.00160
D	0.0429 ± 0.0027	-0.00689 ± 0.00216	-0.01033 ± 0.00273

We therefore subjected several data sets to two other regression models. One model used all data with non-zero light values and considered oxygen metabolism versus the logarithm of light level. The other model used all positive light and oxygen values and considered $1/O_2$ versus 1/light. This latter transformation is analogous to the Burke-Lineweaver plot used in enzyme kinetics and can be used to calculate the parameters of the familiar Michaelis-Menton hyperbolic curve describing metabolic response to a limiting substrate.

With some data sets, one transformation or the other did improve the statistical fit (as measured by the correlation coefficient for the data in their transformed format). Ordinarily the improvement was not dramatic; in many instances the correlations decreased. The linear fit was actually the best in the plurality of cases examined. Some data sets showed a poor linear relation between O_2 and light. Invariably this characteristic proved to be the result of random scatter which was not improved by the transformations. We conclude that the routine descriptive analysis of metabolic response to light in the microcosms is best undertaken by linear regression. We believe that the functional relationship is non-linear but that examination of the data in detail for the functional relationships would require more detailed case-by-case consideration than would ordinarily be justified in routine assays of metabolic responses to variables other than light. Resolution of this question is, of course, fundamental to understanding the energy fluxes within particular ecosystems.

According to an analysis of covariance (Table 3) the regression slopes among the four replicate treatments of Figure 4 do not differ significantly from one another. The regression heights do show significant differences. Inspection of the mean oxygen fluxes adjusted for varying mean light levels demonstrates that treatment C is displaced substantially above the other curves. It has previously been pointed out that the functional relation between light and oxygen flux is probably non-linear. Substantial differences

TABLE 3. ANALYSIS OF COVARIANCE FOR DATA IN TABLE 2 AND FIGURE 4

Line	Treatment	f	Σx^2	Σxy	Σy^2	b	Deviations from regression f	Deviations from regression $\Sigma dy \cdot x^2$	Deviations from regression M.S.
A.	Analysis of Covariance								
1	control period 1	34	6.9322	0.3015	0.01883	0.0435	33	0.005719	
2	control period 2	48	8.2333	0.3583	0.02053	0.0435	47	0.004936	
3	control period 3	19	1.0356	0.0615	0.00492	0.0594	18	0.001264	
4	control period 4	22	5.4767	0.2347	0.01098	0.0429	21	0.000923	
5	within						119	0.012842	0.000108
6	regression coefficient						3	0.000256	0.000085
7	common	123	21.6778	0.9560	0.05526	0.0441	122	0.013098	0.000107
8	adjusted means						3	0.001226	0.000409
9	total	126	23.4467	0.9865	0.05559		125	0.014324	

Significant difference among regression coefficients?

$F_{3,119} = M.S._6/M.S._5 = 0.79$ No difference

Significant difference among regression line heights?

$F_{3,122} = M.S._8/M.S._7 = 3.82$ Significant at P = 0.012

B. Adjusted Mean Y's

$$\bar{Y}_1 - b(\bar{X}_1 - \bar{X}_{tot}) = 0.0191 - 0.0441\ (0.626 - 0.511) = 0.0140$$

$$\bar{Y}_2 - b(\bar{X}_2 - \bar{X}_{tot}) = 0.0178 - 0.0441\ (0.490 - 0.511) = 0.0187$$

$$\bar{Y}_3 - b(\bar{X}_3 - \bar{X}_{tot}) = 0.0127 - 0.0441\ (0.271 - 0.511) = 0.0233$$

$$\bar{Y}_4 - b(\bar{X}_4 - \bar{X}_{tot}) = 0.0183 - 0.0441\ (0.587 - 0.511) = 0.0149$$

in the distribution of light values sampled could be expected to be reflected in the characteristics of the regression equations. We therefore suggest that this analysis of covariance indicates the regression slope to be less sensitive to this non-linearity than the curve heights. Corroboration for this interpretation may be found in the comparison (and obvious bias) between directly calculated 0-light O_2 fluxes and the Y-intercept values. It is further evident that covariance comparisons among light versus O_2 equations should first consider different light treatments as a possible explanation for observed regression differences. This possibility can be unequivocally dismissed only in the situation of between-treatment intercomparisons under identically varying light regimes; some caution with the data should make recognition of this possibility obvious.

Figure 5 and Table 4 compare the pre-stress control treatment of Figure 4a (T1) with two similarly treated tanks (T7, T10) before stress. The analysis of covariance demonstrates that the three replicates do not differ significantly from one another. Note that in this instance the light regimes are identical, because the measurements are simultaneous with one another.

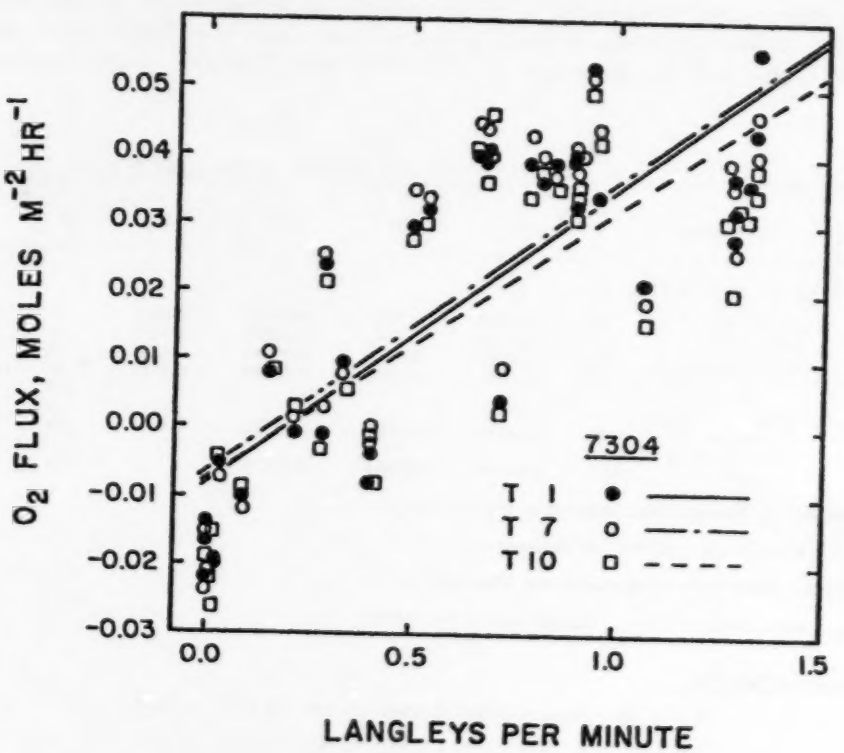

Figure 5. Comparison among replicate treatments under identical light regimes.

TABLE 4. ANALYSIS OF COVARIANCE FOR DATA IN FIGURE 5

Line	Treatment	f	Σx^2	Σxy	Σy^2	b	Deviations from regression f	Deviations from regression $\Sigma dy \cdot x^2$	Deviations from regression M.S.
1	T1	34	6.9322	0.3015	0.01883	0.0435	33	0.005719	
2	T7	34	6.9322	0.3002	0.01949	0.0432	33	0.006494	
3	T10	34	6.9322	0.2777	0.01709	0.0402	33	0.005970	
4	within						99	0.018183	0.000184
5	regression coefficient						3	0.000052	0.000017
6	common	102	20.7967	0.8793	0.05541	0.0423	102	0.018235	0.000179
7	adjusted means						3	0.000180	0.000060
8	total	104	20.7967	0.8793	0.05559		105	0.018415	

Significant difference among regression coefficients?

$F_{3,99}$ = M.S.$_5$/M.S.$_4$ = 0.09 No difference

Figure 6 presents another comparison of replicate treatments. Two tanks (T1, T2) were allowed to develop fouling communities on the bare tank floor and walls over 38 days (24 April through 1 June 1973). Three other tanks (T7, T8, T9) similarly developed over only 22 days. Analyses of covariance for these data are presented in Table 5. The duplicate 38-day treatments are

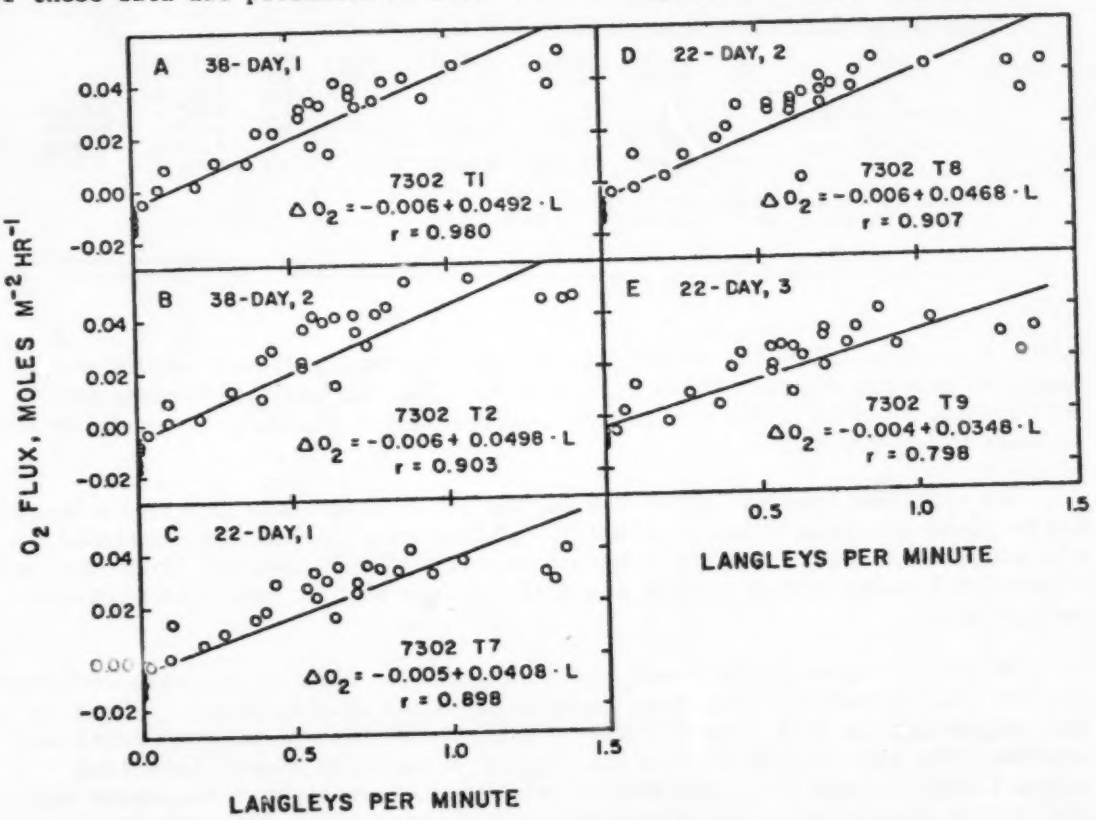

Figure 6. Solar radiation versus oxygen flux treatments discussed in text.

TABLE 5. ANALYSIS OF COVARIANCE FOR DATA IN FIGURE 6

Line	Treatment	f	Σx^2	Σxy	Σy^2	b	Deviations from regression: f	Deviations from regression: $\Sigma dy \cdot x^2$	Deviations from regression: M.S.
A. Comparison of 38-day replicates									
1	T1	38	6.7933	0.3351	0.017200	0.0492	37	0.000666	
2	T2	38	6.7933	0.3373	0.02056	0.0498	37	0.003809	
3	within						74	0.004475	0.000060
4	regression coefficient						1	0.000000	0.000000
5	common	76	13.5867	0.6725	0.03776	0.0495	75	0.004475	0.000060
6	adjusted means						1	0.001740	0.001740
7	total	77	13.5867	0.6725	0.03950		76	0.006215	
Significant differences among regression coefficients? $F_{1,74} = M.S._4/M.S._3 = 0.00$ No difference									
B. Comparison of 22-day replicates									
1	T7	38	6.7933	0.2764	0.01394	0.0408	37	0.002692	
2	T8	38	6.7933	0.3175	0.01803	0.0468	37	0.003191	
3	T9	38	6.7933	0.2372	0.01061	0.0348	37	0.002331	
4	within						111	0.008214	0.000074
5	regression coefficient						2	0.000475	0.000238
6	common	114	20.3800	0.8311	0.04258	0.0408	113	0.008689	0.000077
7	adjusted means						2	0.000280	0.000140
8	total	116	20.3800	0.8311	0.04286		115	0.008969	
Significant differences among regression coefficients? $F_{2,111} = M.S._5/M.S._4 = 3.22$ Significant at P = 0.04									
C. Intercomparison between pooled 38-day treatments and pooled 22-day treatments									
1	T1+T2	77	13.5867	0.6725	0.03950	0.0495	76	0.006215	
2	T7+T8+T9	116	20.3800	0.8311	0.04286	0.0408	115	0.008969	
3	within						191	0.015184	0.000079
4	regression coefficient						1	0.000619	0.000619
5	common	193	33.9667	1.5036	0.08236	0.0444	192	0.015803	0.000082
6	adjusted means						1	0.000230	0.000230
7	total	194	33.9667	1.5036	0.08259		193	0.016033	
Significant differences among regression coefficients? $F_{1,191} = M.S._4/M.S._4 = 7.84$ Significant at P = 0.006									

virtually identical. The triplicate 22-day treatments show somewhat more scatter, and the slopes differ at P = 0.04. The two sets of treatments differ significantly from one another, although the absolute slope difference is only about 0.009.

We conclude from the above two series of analyses that replicate treatments yield generally similar results. The results become less variable as the communities age. Fouling communities started at different times but under otherwise similar circumstances converge towards similar metabolic characteristics.

Figure 5 suggests the basis for still another form of analysis to "remove" the variable metabolic responses associated with variable light. There is the suggestion in that figure that the tank metabolism values may track one another even better than they track light. Presenting direct intertank comparisons (Figure 7) rather than metabolism versus light comparisons supports this suggestion. The simultaneous tank metabolism measurements are highly correlated (r = 0.99), and the functional (or geometric mean) regression lines (after Ricker, 1973) have slopes near + 1.0 and intercepts near 0.0.

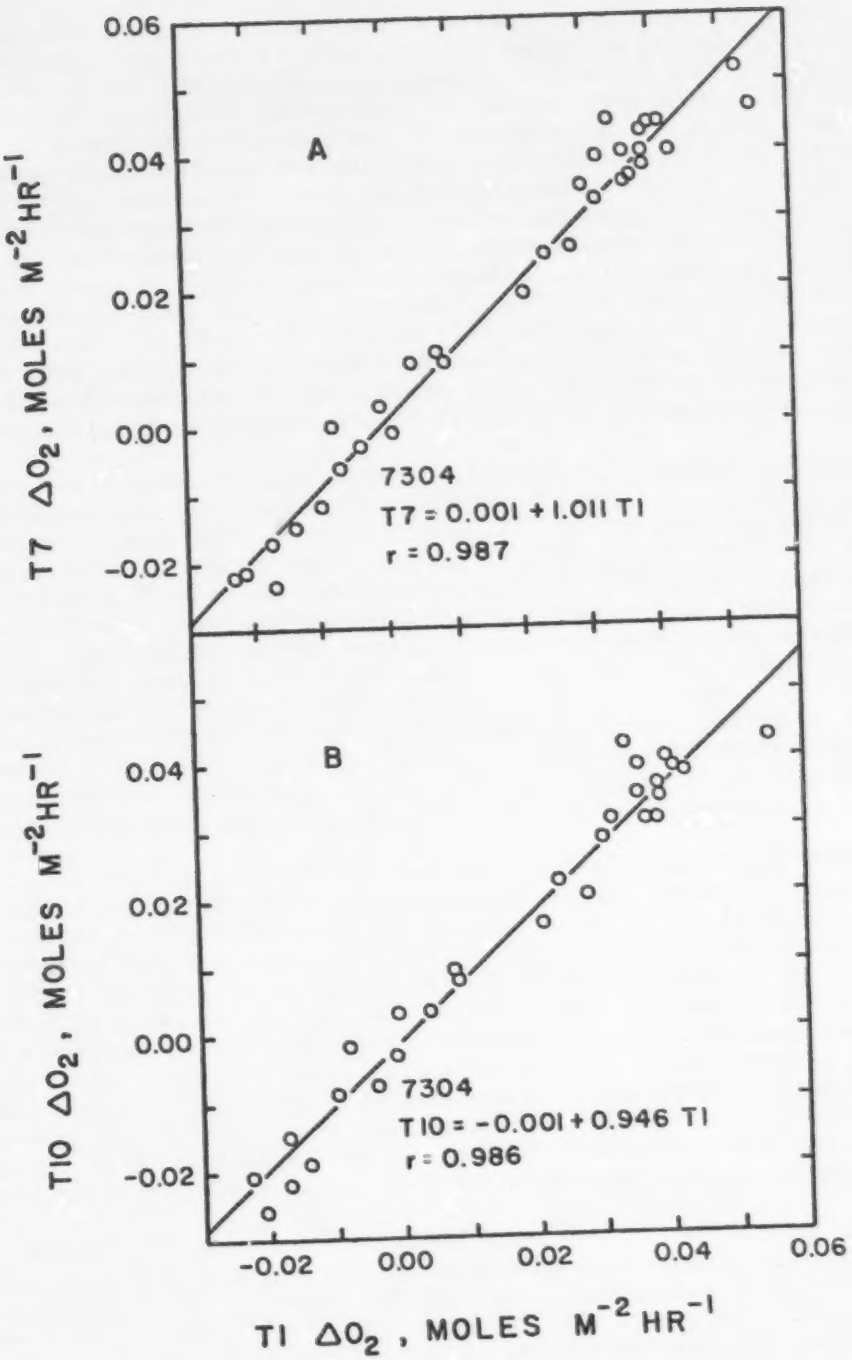

Figure 7. Comparison of replicate tank oxygen fluxes.

Obviously the direct intertank comparisons eliminate metabolic variability which is not explained statistically by the light versus metabolism regression. Both the higher correlations and the ability to make comparisons if (or when) light data are not available make such direct intertank comparisons very useful. A disadvantage of these direct comparisons is that data collected on different days (or otherwise not simultaneously collected) cannot be compared. Moreover, the statistical basis for interpreting regressions on data arrays with rigorously defined independent and dependent variables is more firmly established than is the basis for intercomparison of two dependent variables (Ricker, 1973). With correlations as high as those shown in Figure 7, this latter point is trivial. However, not all such intercomparisons can be expected to show this virtually perfect correlation. When the data sets permit, we generally prefer covariance analysis among data sets for which regressions of metabolism versus light have been calculated as the analysis of primary preference, with the direct comparisons as supplementary analyses.

Let us consider the energetic significance of these light versus metabolism analyses. For the communities represented, the regression slopes are near 0.045 moles O_2 m^{-2} hr^{-1} $langley^{-1}$ minute. This slope term can be reduced to $7.5X10^{-5}$ moles O_2/kcal light. If we assume that one mole of oxygen production equals (to a first approximation) one mole of organic carbon production and that one mole of organic carbon has a caloric value of about 120 kilocalories, then the light to fixed carbon energy conversion efficiency is about 1 percent. Because of light absorption through the water column and because the light measurement includes light outside the photosynthetically active spectrum, this figure underestimates the efficiency with which actually available light is converted to organic carbon. Odum (1971) cites conversion efficiencies of 3 to 5 percent for the net production of intensively cultivated terrestrial crops and only 0.5 percent as an average favorable condition.

Both the relatively high conversion efficiency and the nearly linear relationship between available light and productivity suggest that these fouling communities (and, by extension, other shallow reef benthos communities) are strongly light-limited. Available data for shallow water reef communities (summarized by Smith, 1974) usually yield gross production to respiration ratios near 1.0. Unless the respiration rates of such communities drop concommitantly with production, reef communities at moderate water depths may be heterotrophic. The shift in production with water depth should follow an exponential decay curve in parallel with the vertical pattern of light attenuation. It might be postulated that a shift in community composition with depth could compensate for lower light levels; the high conversion efficiencies suggest that there is little ecological margin for such a shift.

It is evident that the association between extensive development of successful coral reef communities and clear waters is more than coincidental. Gross community production rates near 0.5 moles m^{-2} day^{-1} (Smith, 1974) with solar input of not more than 600 g cal cm^{-2} day^{-1} (Holmes, 1957) imply solar conversion efficiencies similar to the ones we have measured in the microcosms. Waters which are naturally or artificially turbid must significantly depress organic carbon production of reef benthos.

B. Substratum

The composition of reef communities is closely related to the nature of the substratum inhabited by these communities. Organisms living both within the interstices of the substratum and on the substratum surface are influenced by the size of the interstices and by the stability of the substratum. Substratum irregularity alters the surface area, hence the "surface area index" (Dahl, 1973). Substratum composition (e.g. grain size) relates to the ambient environment, and as such is an index of environmental conditions (e.g. water motion, water clarity) which may directly affect the composition of the biota. Coral reef environments can be found effectively spanning the range of possible substratum characteristics, so it is important to quantify reef response characteristics to substratum composition. Artificial (e.g. dredging) or natural (e.g. storms) processes may dramatically alter substratum characteristics through either abrupt change or slow transition.

This section considers the oxygen metabolism of shallow benthos communities in direct response to the nature of the substratum. The experiments explicitly eliminate secondary variables which may be correlated with substratum type but which may affect metabolism independently of substratum. For example, the substrata discussed here (mud, sand, rubble, hard bottom) may be asscciated with particular water motion regimes; and water motion may affect metabolism directly. Mud, especially in shallow water where it is likely to be re-suspended, is often associated with lowered water clarity or depressed salinity--either of which might alter metabolism. The tanks eliminate these secondary effects.

Figure 8 is a series of scatter diagrams of light versus oxygen flux for three microcosm fouling communities. These communities developed on rubble, sand, and mud bottoms for 33 days; community metabolism was then measured on each of eight days over the next 21 days. Figure 8a presents the same data as the diagram in Figure 4. Several facts emerge. First, linear regressions provide relatively good fits for the data from all three communities. Second, despite the good descriptive fit, the true functional relationship is apparently non-linear. This interpretation can be made with most confidence for Figure 8a (rubble). Third, the 0-light intercept is overestimated by the regression lines. Analysis of covariance (Table 6) demonstrates that there are significant differences among the regression slopes. Further analyses demonstrate that the lines for sand and mud (T2, T3) do not differ from one another. The pooled slope for those substrata is 0.0276, and the slope for rubble is 0.0420.

Analysis of covariance allows the comparison of these data with data collected in another experimental series. The data represented in Figure 6a and 6b are for fouling community development over 38 days (about the same time span as the Figure 8 data) on the walls and floor of the microcosms. Because the rubble substratum has already been isolated as significantly different from sand and mud, and because the hard bottom tanks have apparently higher metabolism/light coefficients than the rubble tank, only the rubble and hard bottom are compared.

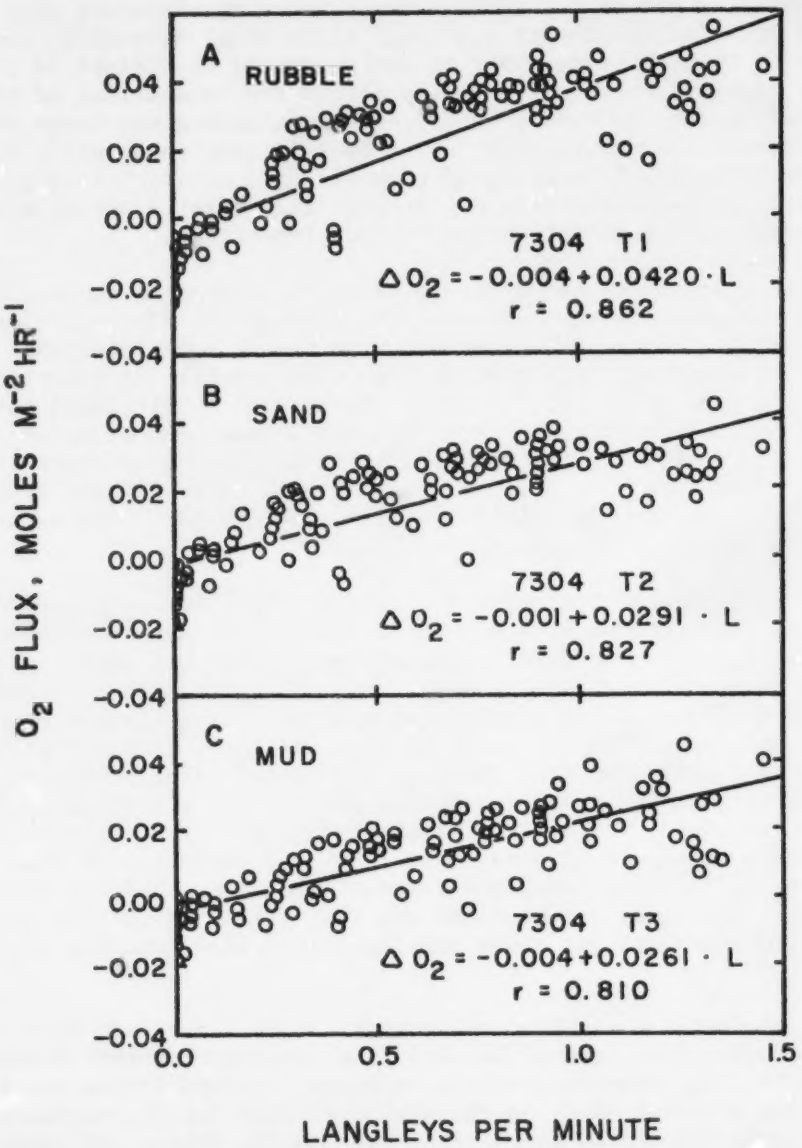

Figure 8. Solar radiation versus oxygen flux of benthos communities developed on various substrata.

TABLE 6. ANALYSIS OF COVARIANCE FOR DATA IN FIGURE 8

Line	Treatment	f	Σx^2	Σxy	Σy^2	b	Deviations from regression f	Deviations from regression $\Sigma dy \cdot x^2$	Deviations from regression M.S.
1	rubble (T1)	126	23.4733	0.9870	0.05583	0.0420	125	0.014333	
2	sand (T2)	126	23.4733	0.6855	0.02928	0.0291	125	0.009261	
3	mud (T3)	126	23.4733	0.6118	0.02430	0.0261	125	0.008356	
4	within						375	0.031950	0.000085
5	regression coefficient						2	0.003367	0.001684
6	common	378	70.4200	2.2842	0.10941	0.0324	377	0.035317	0.000094
7	adjusted means						2	0.003870	0.001935
8	total	380	70.4200	2.2842	0.11328		379	0.039187	

Significant difference among regression coefficients?

$F_{2,375} = M.S._5/M.S._4 = 19.81$ Significant at 0.001

Table 7 demonstrates that the slope of the hard-bottom metabolism curve is steeper than the rubble metabolism curve. Because these experiments were not conducted simultaneously, this comparison must be treated with some caution. Nevertheless, the two experiments suggest a metabolic progression with respect to substratum: hard > rubble > sand ≈ mud. The data at hand suggest that the ratios of metabolic response are 1.8:1.5:1.1. Rubble and hard bottom are capable of supporting 50 to 80 percent higher metabolic rates than sand or mud as a direct consequence of substratum type.

TABLE 7. ANALYSIS OF COVARIANCE FOR DATA IN FIGURE 6A, B AND FIGURE 8A

Line	Treatment	f	Σx^2	Σxy	Σy^2	b	Deviations from regression f	Deviations from regression $\Sigma dy \cdot x^2$	Deviations from regression M.S.
1	rubble	126	23.4733	0.9870	0.05583	0.0420	125	0.014333	
2	hard	77	13.5867	0.6725	0.03950	0.0495	76	0.006215	
3	within						201	0.020548	0.000102
4	regression coefficient						1	0.000477	0.000477
5	common	203	37.0600	1.6594	0.09533	0.0448	202	0.021025	0.000104
6	adjusted means						1	0.000043	0.000043
7	total	204	37.4278	1.6714	0.09571		203	0.021068	

Significant difference between regression coefficients?

$F_{1,201} = M.S._4/M.S._3 = 4.68$ Significant at 0.03

The intercomparison of metabolic rates can be effected more directly for the mud, sand, and rubble substrata. Figure 9 presents scatter diagrams of rubble versus sand and rubble versus mud plots and the functional or geometric mean regression coefficients (after Ricker, 1973). If those slopes are used to establish the rubble:sand:mud metabolic ratios, the results are 1.5:1.1:1—virtually identical with the covariance approach.

The explanation for enhanced metabolism on rubble or hard-bottom substrate is not entirely clear. Experimental design rules out water motion or

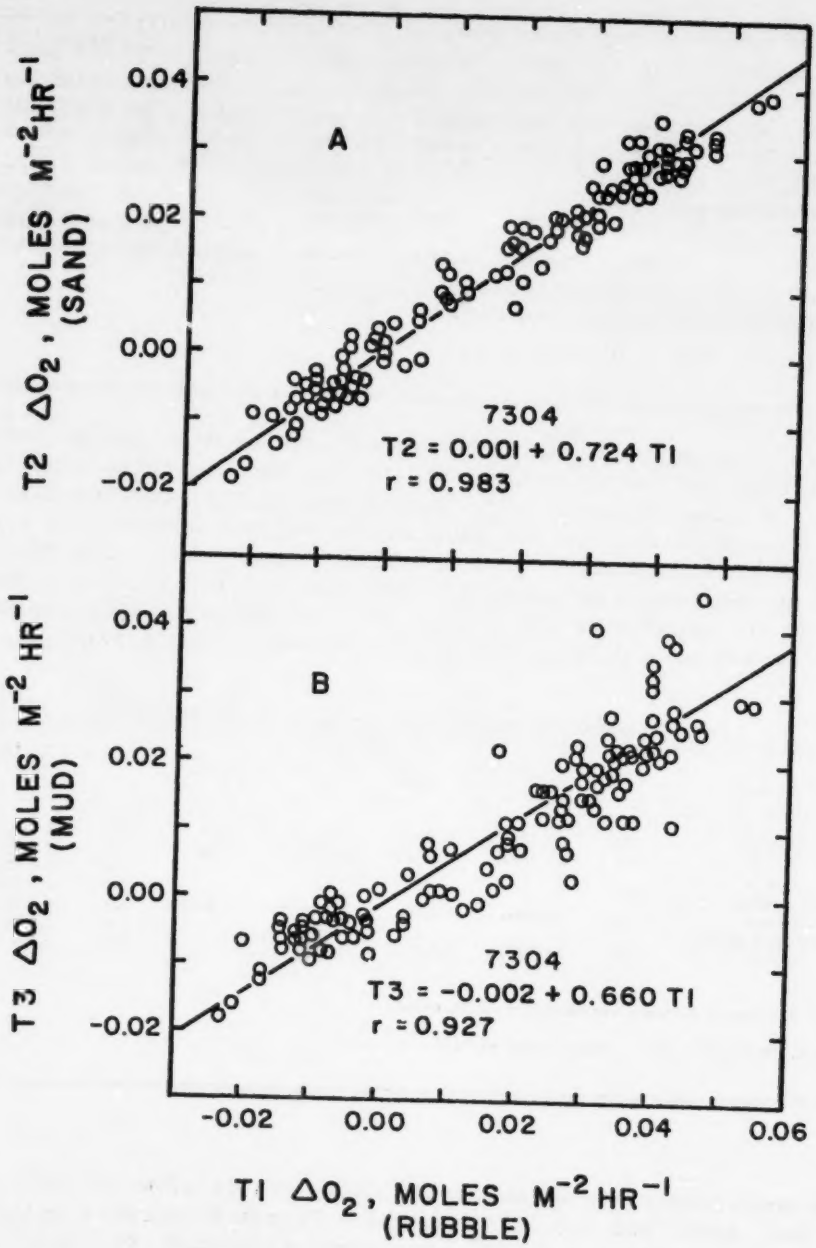

Figure 9. Direct comparison of oxygen flux on rubble versus sand and mud substrata.

water clarity. Physical stability may be a partial answer, inasmuch as bioturbation of sand and mud could disrupt the algal communities on those substrata. Increased surface area for algal growth could explain the difference between the rubble and the sand-mud comparison; this explanation is not consistent with the low-surface area hard bottom being the most active substratum. There may be an effect associated with reflectivity of the surfaces, since light does appear to be a limiting resource. Bacterial respiration in the sediments is yet another possible explanation. Without having an entirely satisfactory explanation for this phenomenon, we report the existence of a direct link between type of substratum and metabolic activity in these shallow tropical benthos microcosms.

C. Nutrients

Coral reefs are ordinarily considered to be products of low-nutrient conditions, and to cycle available nutrients effectively. Kaneohe Bay, Hawaii (the site of our microcosm facility), is subjected to high levels of nutrient loading, primarily from sewage discharge. We therefore wished to determine whether reef communities in Kaneohe Bay were still limited by available nutrients or were nutrient-saturated.

Fouling communities were allowed to develop in each of three tanks. The experimental communities also included three grazing herbivorous fishes (*Acanthurus triostegus*) in each tank. After the fouling communities were inoculated with unfiltered water from the bay, bay water flow to one tank was cut by 30 percent (i.e. by 3 liters min^{-1}). Total flow was brought back to 10 liters min^{-1} by the addition of water from a saltwater well. Salinity of that well water is virtually identical to salinity of bay water ($\approx$ 35 °/oo), but nutrient levels of the well water are substantially higher than bay water (Table 8). Thus, there were two replicate bay water communities compared with one community enriched with well water.

TABLE 8. COMPARISON OF TYPICAL DISSOLVED INORGANIC NUTRIENT LEVELS IN BAY WATER DRAWN THROUGH MICROCOSM FACILITY WITH SEAWATER WELL

	PO_4	NO_3	NH_4	Total inorganic N
	µmoles/liter			
Bay water	0.5	0.6	1.6	2.2
Well water	1.2	5.0	5.0	10.0
30% well + 70% bay	0.7	1.9	2.6	4.5
Well/bay ratio	2.4	8.3	3.1	4.5
(70% bay + 30% well)/ bay ratio	1.4	3.2	1.6	2.1

Figure 10 illustrates metabolic rate as a function of light in the three tanks. The analysis of covariance is presented in Table 9. It is evident that the 30 percent bay water addition boosted community metabolic response to light by about 43 percent and that the two replicate treatments receiving

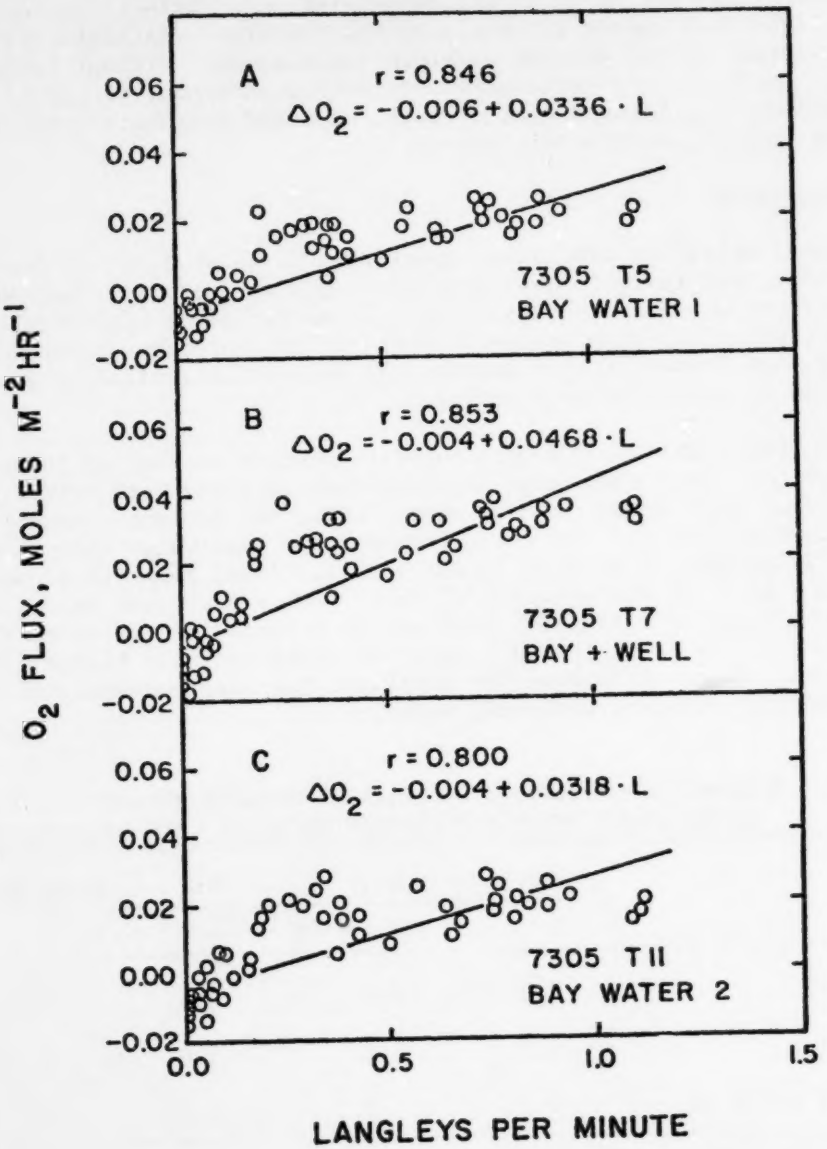

Figure 10. Solar radiation versus oxygen flux for "low nutrient" (bay water) and "high nutrient" (bay + well water) treatments.

TABLE 9. ANALYSIS OF COVARIANCE FOR DATA IN FIGURE 10

Line	Treatment	f	Σx^2	Σxy	Σy^2	b	Deviations from regression f	Deviations from regression $\Sigma dy \cdot x^2$	Deviations from regression M.S.
1	bay water 1	68	7.8533	0.2638	0.01237	0.0336	67	0.003509	
2	bay + well	68	7.8533	0.3670	0.02359	0.0467	67	0.006439	
3	bay water 2	68	7.8533	0.2490	0.01234	0.0317	67	0.004447	
4	within						201	0.014395	0.000072
5	regression coefficient						2	0.001051	0.000525
6	common	204	23.5600	0.8798	0.04830	0.0374	203	0.015446	0.000076
7	adjusted means						2	0.001410	0.000705
8	total	206	23.5600	0.8798	0.04971		205	0.016856	

Significant difference among regression coefficients?

$F_{2,201} = M.S._5/M.S._4 = 7.29$ Significant at 0.001

bay water tracked one another well. Figure 11 is a plot of O_2 metabolism in the bay + well tank versus O_2 in the two bay tanks. This direct fit yields a well/bay regression slope of 1.36--very close to the value of 1.43 inferred from the covariance analysis. The metabolic enhancement by well water is close to the proportional increase in phosphorus loading (Table 8).

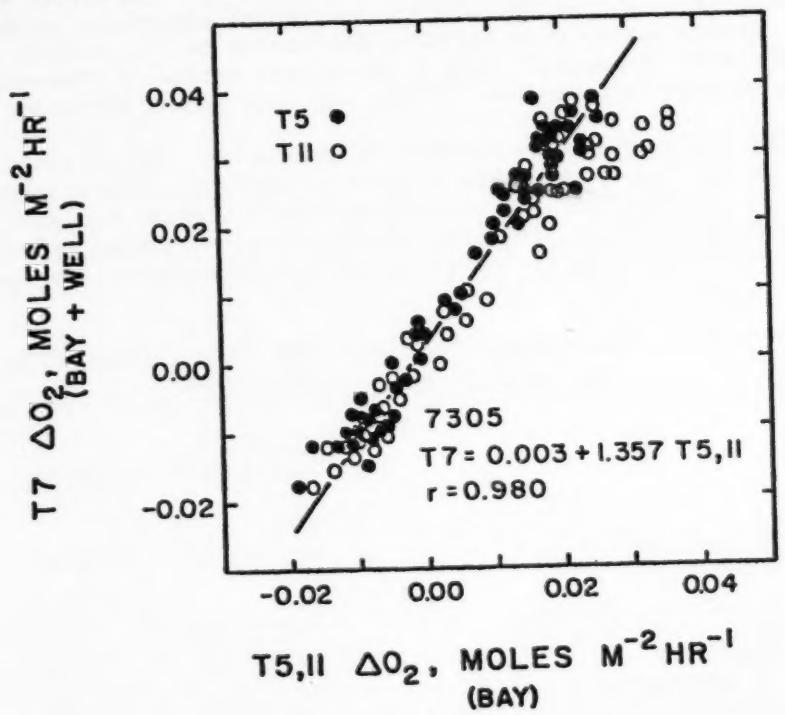

Figure 11. Direct comparison of "low nutrient" (bay water) versus "high nutrient" (bay+well water) treatments.

There are several conclusions to be drawn from these analyses. The microcosm benthos--and by inference the shallow benthos of Kaneohe Bay--increase their metabolic rate when nutrient levels are raised. Therefore, increased nutrient loading in the bay would result in higher benthos production. Conversely, lowering the nutrient loading should decrease production. Moreover, the microcosms prove from these preliminary observations to be a useful tool for examination of the effects of nutrient loading. At this writing, this set of conclusions has been coupled with the fact that a major nutrient input to Kaneohe Bay is being removed (diversion of sewage), to expand this observation into a more detailed analysis of the effects of nutrient loading on reef communities. Included in that analysis is an assessment both of the generalized effects and a more specific analysis of the individual roles of nitrogen and phosphorus.

D. Salinity

Reef ecosystems are ordinarily restricted to areas of near-oceanic salinity (≈ 35 °/oo), although there are reefs which experience moderate departures from this value. Freshwater "kills" from rains and runoff have been reported. Reef communities are frequently suppressed in the vicinity of streams. The tolerance of reef communities for salinities between 0 °/oo and 35 °/oo has not been well defined. We therefore conducted an experiment with two primary purposes. Approximately what salinity depression begins to kill conspicuous parts of the reef community? Does a measurable metabolic response occur at salinities intermediate between 35 °/oo and this lethal threshold?

Fouling communities were allowed to develop on rubble, sand, and mud substrata in the microcosms. The results within the substratum types were qualitatively similar, but between-salinity differences were most conspicuous on the rubble substratum. Only the results from the rubble experiments are reported here. Organisms listed in Table 10 were added to the fouling

TABLE 10. ORGANISMS INTRODUCED INTO FOULING COMMUNITIES FOR SALINITY STRESS EXPERIMENTS

ALGAE	CRUSTACEANS
Acanthopora spicifera	*Chiridota hawaiiensis*
ECHINODERMS	FISHES
Opheodesoma spectabalis	*Acanthurus triostegus*
Holothuria monocaria	
COELENTERATES	
Zoanthus pacificus	
Pocillopora damicornis	
Porites compressa	

communities. These organisms are all typical of the fringing reef communities of Kaneohe Bay. It was determined that the organisms added were themselves insufficient to alter the microcosm community metabolism significantly. Hence, observed metabolic responses--if any--would be that of the fouling communities. These large organisms were simply added as indicators of stress.

Table 11 reports the chronology of salinity alteration. The initial salinity reductions (to 29 and 25 °/oo) imposed no measurable metabolic response or lethal effect on the microcosms, so the stresses were relaxed and then applied more severely (to 22 and 16 °/oo). The experiment was performed as described, with the assumption that the initial salinity reductions had no effect. This experiment should be repeated in a manner which precludes the need for such an assumption.

TABLE 11. CHRONOLOGY OF SALINITY STRESS EXPERIMENTS

Date (day of year)	Day of Experiment	Event
11 June 1973 (162)	0	Start experiment
14 July 1973 (195)	33	Retain tanks 1,2,3 as controls Lower tanks 7,8,9 to 25 °/oo Lower tanks 10,11,12 to 29 °/oo
19 July 1973 (200)	38	Return all tanks to 35 °/oo
24 July 1973 (205)	43	Retain tanks 1,2,3 as controls Lower tanks 7,8,9 to 22 °/oo Lower tanks 10,11,12 to 16 °/oo
25 July 1973 (206)	44	Return all tanks to 35 °/oo
17 August 1973 (229)	67	Terminate experiment

Only the lowest salinity (16 °/oo) killed a substantial fraction of the reef organisms. All organisms listed in Table 10 as well as some of the infauna which had entered the microcosms as fouling organisms were affected during the 24-hour exposure. The coelenterates and echinoderms were all killed by the lowest salinity; the other animals recovered once the salinity was returned to normal. Algal survival could not be assessed by visual inspection, so is not reported here. Figure 12 and Table 12 show the results of the metabolic measurements. Over the entire salinity range employed, there was no significant variation in metabolic response.

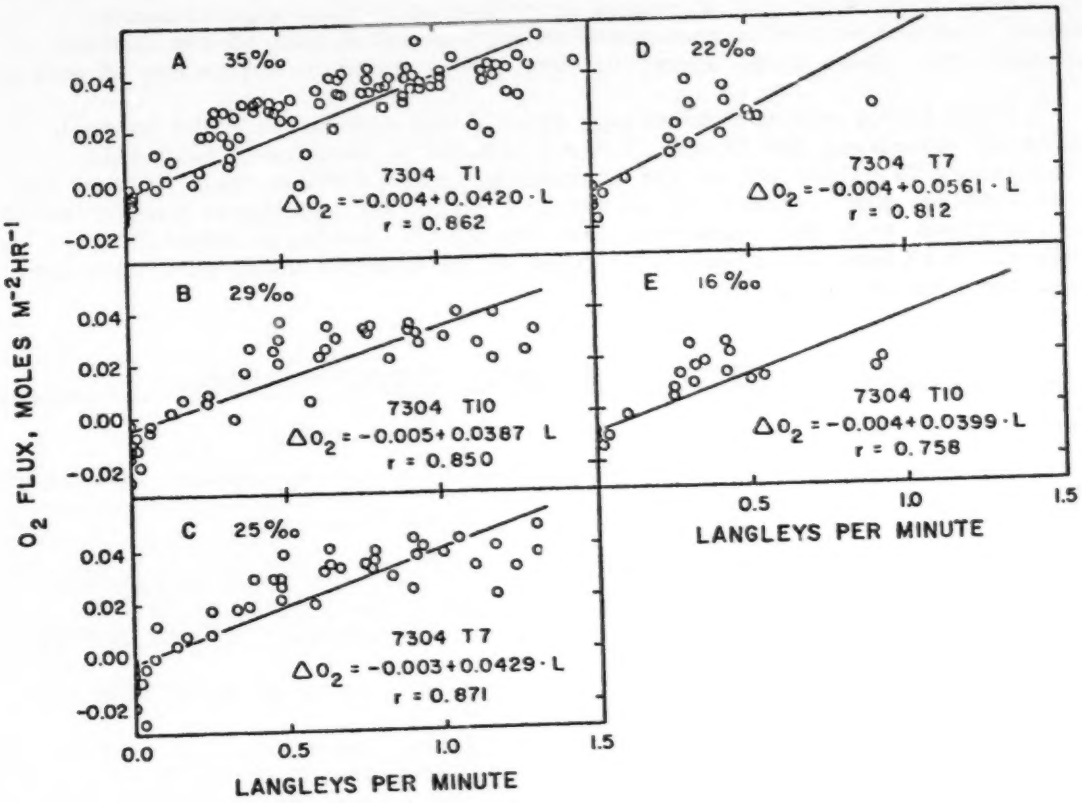

Figure 12. Solar radiation versus oxygen flux under various salinity regimes.

There are two primary conclusions to be drawn from this surprising result. Although salinities below 22 °/oo (i.e. below 63 percent of ambient salinity) did prove detrimental to the conspicuous reef organisms that were added to the microcosms, these organisms were minor in the total metabolic activity of the reef community. If we assume that the effects of lowered salinity were ecologically significant--despite their relatively minor "energetic" importance--then we conclude that there was not a community metabolic harbinger to damage from salinity depression. Although it has been demonstrated that community metabolism is responsive to other perturbations, metabolism of reef benthos communities is not sensitive to sublethal variations in salinity.

TABLE 12. ANALYSIS OF COVARIANCE FOR DATA IN FIGURE 12

Line	Treatment	f	Σx^2	Σxy	Σy^2	b	Deviations from regression f	Deviations from regression $\Sigma dy \cdot x^2$	Deviations from regression M.S.
1	35 °/oo	126	23.4711	0.9869	0.05583	0.0420	125	0.014332	
2	29 °/oo	48	8.2333	0.3175	0.01696	0.0387	47	0.004715	
3	25 °/oo	48	8.2333	0.3521	0.01984	0.0429	47	0.004781	
4	22 °/oo	19	1.0356	0.0582	0.00497	0.0561	18	0.0001695	
5	16 °/oo	19	1.0356	0.0413	0.00287	0.0399	18	0.001223	
6	within						255	0.026746	0.000105
7	regression coefficient						4	0.000314	0.000079
8	common	260	42.0089	1.7561	0.10047	0.0418	259	0.027060	0.000104
9	adjusted means						4	0.000566	0.000141
10	total	264	43.8400	1.8197	0.10316		263	0.027626	

Significant differences among regression coefficients?

$F_{4,255} = M.S._7/M.S._6 = 0.75$ No difference

E. Biological Manipulation

The analyses presented so far have taken little note of the biological composition of the microcosm communities. The communities have been considered only as fouling communities, with reference to some specific community alterations common to all treatments. Such a presentation has been deliberate. The philosophy of the analyses and of the presentation has been that community metabolism is a) easy to measure, b) sensitive to variation in (some) external forcing functions, and c) relatively insensitive to uncontrolled variations in community structure associated with random recruitment.

The previous sections have established the validity of these points. Is community metabolism sensitive to direct, deliberate manipulation of biological composition? This question is the subject of the present section.

Three tanks were set up with a sand substratum and allowed to develop a fouling community. Three herbivorous fishes (*Acanthurus triostegus*) were added to one tank; three herbivorous fishes of a different genus (*Scarus* sp.) were added; there were no additions to the third tank. The fish biomass in the two tanks with fishes was roughly comparable. These two herbivores have significantly different feeding strategies. *Acanthurus* feeds by biting off strands of algae; hence this fish is relatively effective with larger algae. *Scarus* feeds by scraping rock surfaces and is most effective with a fine algal turf or endolithic algae. Separate experiments demonstrated that the respiration of the fishes themselves was a negligible contribution to the total microcosm community metabolism.

Figure 13 is a plot of oxygen metabolism versus light for the three treatments, and Table 13 is the covariance analysis. Community metabolism is sensitive to fish grazing pressure; given comparable biomasses (and, one presumes, roughly comparable metabolic demands) of herbivorous fishes, the communities are not shown to be sensitive to feeding strategy. Rubble communities with and without three *Acanthurus* are also metabolically distinct (Table 14), offering further validation that this kind of biological manipulation affects metabolism.

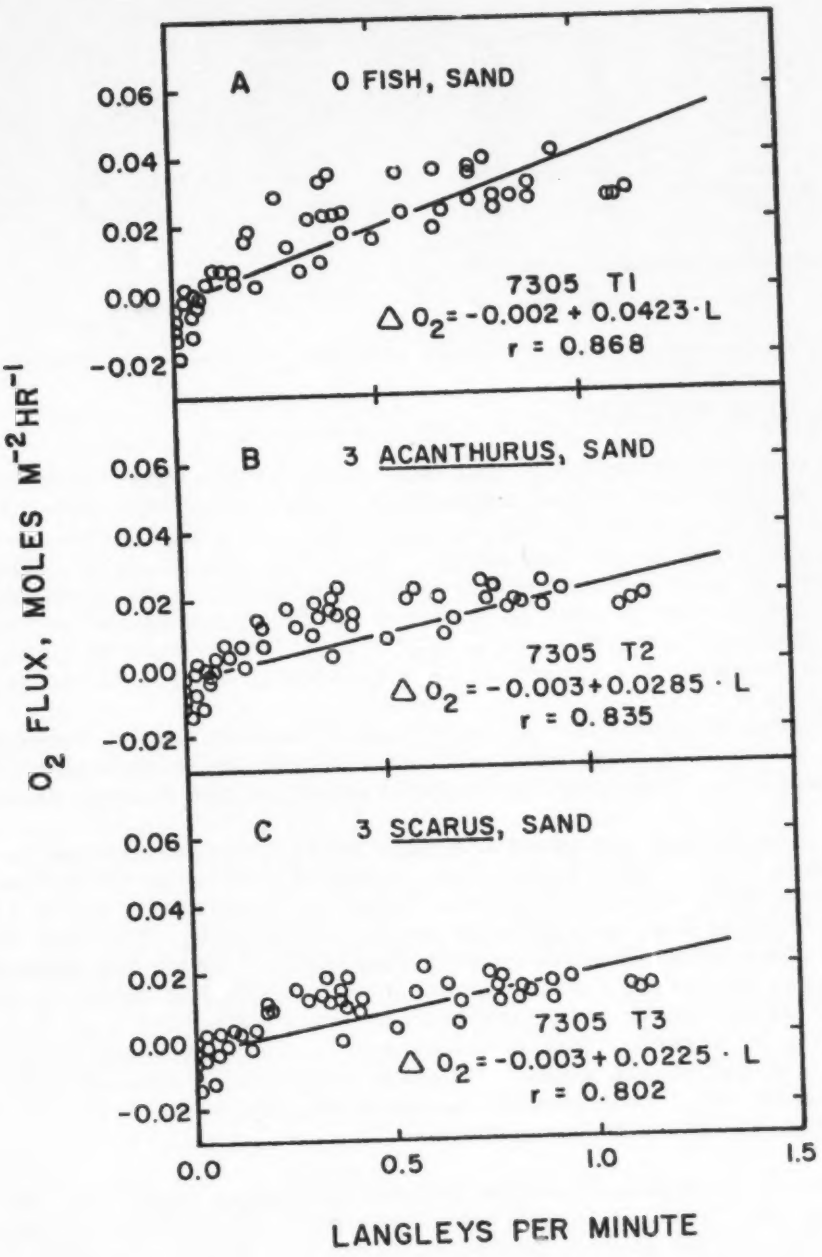

Figure 13. Solar radiation versus oxygen flux in tanks with and without herbivorous fishes.

TABLE 13. ANALYSIS OF COVARIANCE FOR DATA IN FIGURE 13

Line	Treatment	f	Σx^2	Σxy	Σy^2	b	Deviations from Regression f	$\Sigma dy \cdot x^4$	M.S.
1	0 fish, sand	68	7.8533	0.3314	0.01858	0.04230	67	0.004593	
2	3 *Acanthurus*, sand	68	7.8533	0.2230	0.00908	0.0285	67	0.002750	
3	3 *Scarus*, sand	68	7.8533	0.1757	0.00610	0.0225	67	0.002169	
4	within						201	0.009512	0.000047
5	regression coefficient						2	0.001622	0.000811
6	common	204	23.5600	0.7301	0.03376	0.0310	203	0.011135	0.000055
7	adjusted means						2	0.001430	0.000715
8	total	206	23.5600	0.7301	0.3519		205	0.012565	
Repeat lines 4-8 for *Acanthurus* v. *Scarus*									
4'	within						134	0.004919	0.000037
5'	regression coefficient						1	0.000142	0.000142
6'	common	136	15.7067	0.3987	0.01518	0.0255	135	0.005061	0.000037
7'	adjusted means						1	0.000090	0.000090
8'	total	137	15.7067	0.3987	0.01527		136	0.005151	

Significant differences among regression coefficients?

$F_{2,201} = M.S._5/M.S._4 = 17.26$ Significant at P = 0.001

Repeat, testing *Acanthurus* v. *Scarus*

$F_{1,134} = M.S._{5'}/M.S._{4'} = 3.84$ No difference at ? = 0.05

The next comparison involves a complex reef rubble community (transplanted from the reef flat nearly *in toto* and then allowed to stabilize), a sterile rubble community to which two species of grazing echinoids (5 specimens each of adult *Tripneustes gratilla* and *Echinometra mathaei*) were added, and the duplicate rubble/*Acanthurus* communities previously considered. Analysis of covariance (Table 15) demonstrates no significant difference among the regression slopes. The variety of choices of grazers, from fishes to large invertebrates to cryptic invertebrates, has imposed similar effects on community metabolic response to light. Apparently community metabolism is sensitive to grazing pressure but not to the quality of grazing. Either by chance or adaptive response, the communities have shown very similar responses despite our approach to adding the herbivore level in a trophic pyramid.

TABLE 14. ANALYSIS OF COVARIANCE, NO FISH VERSUS FISH, RUBBLE

Line	Treatment	f	Σx^2	Σxy	Σy^2	b	Deviations from regression f	$\Sigma dy \cdot x^2$	M.S.
1	0 fish, rubble	137	15.7067	0.7162	0.04541	0.0456	136	0.012749	
2	3 *Acanthurus*, rubble	137	15.7067	0.5128	0.02477	0.0327	136	0.008030	
3	within						272	0.020779	0.000076
4	regression coefficient						1	0.001318	0.001318
5	common	274	31.4133	1.2290	0.07018	0.0390	273	0.022097	0.000081
6	adjusted means						1	0.002020	0.002020
7	total	275	31.4133	1.2290	0.07220		274	0.024117	

Significant differences between regression coefficients?

$F_{1,272} = M.S._4/M.S._3 = 17.34$ Significant at P = 0.001

There does appear to be a substantially lower net production in the reef rubble community than in the two simpler communities (Table 15b). The three communities respond similarly to varying light, so we assume that the lower net production represents a higher community respiration rate.

TABLE 15. ANALYSIS OF COVARIANCE, REEF RUBBLE V. ECHINOIDS V. FISH

Line	Treatment	f	Σx^2	Σxy	Σy^2	b	Deviations from regression f	Deviations from regression $\Sigma dy \cdot x^2$	Deviations from regression M.S.
A.	Analysis of Covariance								
1	reef rubble	68	7.8533	0.3054	0.01599	0.0390	67	0.004111	
2	sterile rubble + echinoid	68	7.8533	0.2542	0.01243	0.0324	67	0.004200	
3	sterile rubble + fish	137	15.7056	0.5128	0.02477	0.0327	136	0.008029	
4	within						270	0.016340	0.000061
5	regression coefficient common	273	31.4122	1.0724	0.05319	0.0342	2	0.000236	0.000118
6	adjusted means						272	0.016576	0.000061
7	total	275	31.4122	1.0724	0.05389		2	0.000700	0.000350
8							274	0.017276	

Significant differences among regression coefficients?

$F_{2,270} = M.S._5/M.S._4 = 1.94$ No difference at P = 0.10

B. Adjusted mean Y's ± 95% confidence interval

$\bar{Y}_1 = 0.0024 \pm 0.0019$

$\bar{Y}_2 = 0.0069 \pm 0.0019$

$\bar{Y}_3 = 0.0052 \pm 0.0013$

One might anticipate, *a priori*, that the effects of grazing pressure on reef metabolism would have been different from those that we observed. In some situations, cropping seems to accelerate growth; that proves not to be true for reef community metabolism, although it might be the case for the metabolic rate normalized per unit biomass rather than per unit area. This latter normalization is difficult (in fact, apparently impossible without at least partial destruction of the community).

We conclude that community metabolism is indeed sensitive to biological manipulations within the microcosms. We suggest that there may be relatively broad limits of herbivore food uptake requirements over which the community metabolism adjusts to a constant rate. A sufficiently low herbivore food demand apparently does allow the community metabolism to increase, and an excessive herbivore food demand will result in eventual starvation. Although not explicitly examined by these analyses, we would anticipate analogous patterns among carnivores and detritivores.

F. Conclusions

In the preceding sections we have examined the metabolic responses of shallow tropical benthos communities to five forcing functions: light, substratum, nutrient loading, salinity, and direct manipulation of the biological community. The first three forcing functions may be treated conceptually as limiting resources, with light being a primary limitation which has not been controlled in these experiments. Salinity depression was investigated as a

direct stress imposed upon the community. Manipulation of community structure and, inferentially, metabolic similarities in apparently replicate treatments provide insight into the ability to extrapolate from the metabolic characteristics of such simplified simulations of nature to reefs under natural conditions.

The manipulations and replications demonstrate that the communities are sensitive to biological alterations but that the sensitivity is only between distinctly different communities. Treated similarly, the communities converge towards similar metabolic rates. Moreover, complexity of the manipulated communities does not appear to be a major factor in the metabolic response characteristics. On this basis, we may extrapolate from the microcosms to the real world.

Limiting resources in the microcosms are strongly reflected in their metabolic responses. Light, substratum characteristics, and nutrient loading all impose substantial and quantifiable metabolic responses on the microcosm communities. We conclude that any alteration of these variables will impose effects on reef communities. It is likely that, as low-level chronic perturbations, these variables are altered more frequently in tropical nearshore ecosystems than other variables. The data presented here only begin to reduce our ignorance about metabolic responses to these variables; far more work is needed to quantify these responses adequately. Information on light response is of primary importance and accumulates most rapidly in an experimental facility such as we describe, because all experiments so-conducted will contain light as an "uncontrolled" independent variable. By such treatment in outdoor microcosms, we are able to achieve natural levels of intensity and natural photoperiod. We are continuing to define reef benthos community responses to nutrient loading. Metabolic responses to substratum alteration remain inadequately described.

The remaining variable which we have examined is salinity. This variable is also frequently altered in nearshore environments. Reef communities have an unexpected resilience to salinity depressions. Neither structural nor metabolic responses to depressed salinity occurred in the microcosms until salinity was reduced below 22 °/oo. Thus, the deleterious effects to reefs associated with lowered salinity may be attributed to extreme salinity depression (probably largely in the form of freshwater lenses floating on the seawater) or to materials introduced with the fresh water (sediment, nutrients, toxins). Salinity depression does not appear to be a major chronic sublethal stress on reef communities.

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APPENDIX

DESIGN, CONSTRUCTION, AND OPERATION OF SHALLOW TROPICAL BENTHOS MICROCOSM FACILITIES

A. General

Two microcosm facilities were developed during the course of this program. These facilities are outdoor constructions designed to simulate environmental characteristics typical of shallow tropical benthic settings. The prototype (Figure 1) is at the Hawaii Institute of Marine Biology (HIMB) and draws water from protected, organic-rich Kaneohe Bay, Hawaii. This facility evolved gradually. All experiments described in this report were performed at the HIMB facility. The Naval Undersea Center (NUC, now the Naval Ocean Systems Center) used the HIMB experiences to design and establish an analogous facility on the clean-water open coast of nearby Ulupau Head. Experiments began there near the termination of the contract period covered by this report.

Despite the proximity of the two locations to one another (Figure A-1) and the similarity of "operating philosophy" at the two facilities, site-specific physical, chemical, and biological considerations provide useful insight into the design and operation of such microcosm facilities. Henderson *et al.* (1976) describe the NUC facility in some detail and provide results from preliminary inter-calibration experiments. This appendix presents design, construction, and operating considerations consistent with the two localities. Inasmuch as design detail must inevitably be site-specific and "state-of-the-art" specific, plans and layout are not presented in any detail here.

The primary operating constraint at HIMB is associated with biological fouling. Large numbers of marine organisms settle throughout the seawater system and capitalize on abundant particulate organic food to grow rapidly, clog pipes, and restrict water flow. The system was designed and constructed to minimize this problem in a manner which in no way impinges upon biological activity within the microcosms themselves. At NUC, the primary operational hurdle was associated with inexpensively securing a seawater system which could reliably draw ocean water from beyond a surf zone where breaking winter waves commonly exceed 3 meters in height for days to weeks at a time.

We describe the microcosm components according to the following divisions:

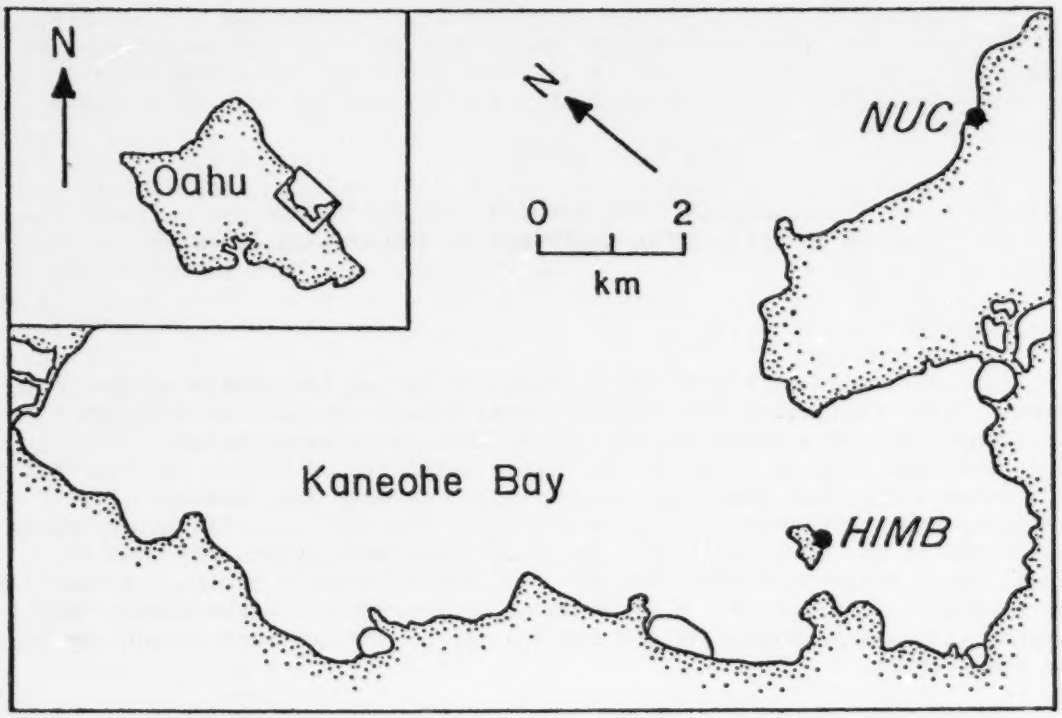

Figure A-1. Map showing locations of HIMB and NUC microcosm facilities.

a. seawater supply and distribution system;

b. microcosm aquaria and associated devices for environmental modification;

c. automatic sampling and data acquisition system.

B. Seawater Supply and Distribution System

The microcosms must be supplied with an uninterrupted flow of seawater. The water should be free of uncontrolled contaminants (e.g. fresh water or sediment from storm runoff). Delivery of water should exceed total anticipated flow through the microcosms by at least 50 percent, so that there is an adequate supply for overflow from constant head tanks, for holding tanks, and so forth. An adequate system should meet the following criteria:

1. Inertness of material--All surfaces coming in contact with water to be delivered to the system must be made of non-toxic plastic, fiberglass, or titanium. All wetted surfaces should be conditioned with running seawater

for several weeks to leach out any residual solvents or other possible toxins before experimental organisms are added. Polyvinyl chloride (PVC) pipes, valves, and other fittings are acceptable, and PVC is particularly easy to use and modify. A single toxic (e.g. copper) fitting among many square meters of otherwise inert materials may prevent the survival of delicate organisms (e.g. corals).

2. Reliability--Microcosm experiments may run for months and are vulnerable to interruptions in seawater delivery. The entire delivery system should be constructed in duplicate (see below, on cleaning) so that there is adequate system redundancy in the event of system failure. The primary pump for the facility is likely to be electrical; there should be an auxiliary generator or a gasoline-driven pump in the event of power failure. The auxiliary system should be periodically tested according to a routine maintenance schedule. There should be an alarm system which operates independently of the electrical system and which is tripped by any interruption in water flow. The alarm itself should also be checked regularly.

3. Cleaning--The distribution system should be designed for ease of cleaning. If the entire system (including pumps) is installed in duplicate, then one system can be shut down for cleaning while the other system is operational. Periodic cleaning minimizes the problem of fouling. Water allowed to stagnate in the closed system will kill newly-settled larvae. This system is then backflushed, to avoid discharge of anoxic water, sulfides, and organic materials into the microcosms. To the extent possible, straight delivery lines should be used instead of ones with many bends, (the straight lines also improve flow characteristics). Open bends should be used rather than tight elbows. Each straight section of pipe should be directly accessible via cleaning ports.

4. Flexibility--In order to accommodate modifications towards differing experimental purposes, facility design should be flexible. Light fiberglass tanks and plastic plumbing are particularly amenable to modification. Sufficient open working area, seawater delivery, drain systems, fresh water, and electrical power should be provided so that facilities can be expanded and modified. In the HIMB system, groups of three microcosms are served by a single inlet headbox. The NUC system allows single or multiple microcosm service by each inlet headbox. The latter arrangement is preferable.

5. Environmental modifications--Water composition can be varied by choice of water source (fresh water, bay water, well water), by manipulation before the water enters the head boxes, by modification in the head boxes (or inlet lines), and by direct manipulation within the tanks. Natural, uncontrolled variations in water quality should be minimized by choice of water intake sites. Care should be taken to see that the microcosms are not shaded by buildings or other obstructions at any time.

6. Plumbing characteristics--Pipe diameters to serve the microcosm system can be calculated from an estimate of the maximum height to which the water must be delivered, the total length of pipe in the distribution system, the flow rates to be maintained, and the pump characteristics. Engineering handbooks or plumbing experts should be consulted for detail, with some

allowance made for fouling. Our facility delivers approximately 400 liters/minute, to a maximum delivery height of 5 meters (an aeration tower). The water flows through approximately 10 meters of pipe before it is delivered to the microcosms. Minimum pipe diameters in the delivery system are 1-1/4 inches. Valves are not used to regulate water flow. Instead, standpipes maintain constant water levels and allow excess water to spill into drains. Flow rates are controlled by head differences between constant-head reservoirs and overflow pipes.

Major considerations about the pump are: 1) that it be capable of delivering the required flow to the maximum elevation in the system; 2) that it be reliable under conditions of constant operation; 3) and that all wetted surfaces be made of non-toxic materials. It is desirable to have two primary pumps (in addition to the auxiliary). One pump can be in operation while the other is down for routine maintenance or repair.

We have used centrifugal pumps. Most small planktonic organisms entrained in the flow survive passage through the pump, although recent studies suggest that larger and more delicate holoplankton and meroplankton are killed.

7. Water characteristics--The water used for most experiments at HIMB is drawn from a depth of 2 meters adjacent to a reef slope in Kaneohe Bay. The water is rich in plankton and organic detritus, so a fouling community develops rapidly. This biological fouling necessitates the double seawater distribution system which has been described. Inlet screens must be exchanged and cleaned fortnightly. A second water source at HIMB is a seawater well. The salinity of that water is very similar to bay salinity. There are other substantial chemical and biological differences between the two water sources. Larvae and organic detritus are effectively filtered from the well water. The only organisms associated with the well water are diatoms which appear to be introduced as airborne contaminents. The inorganic nutrient level of the well water is substantially higher than that of the bay (Table 8). The well water is anoxic and rich in hydrogen sulfide. It is aerated by being flowed as a "film" across rippled plastic roofing material before being delivered to the microcosms. That treatment is sufficient to remove the H_2S and bring the O_2 concentration to saturation.

At the NUC facility, water is drawn from a natural sump at the seaward edge of the reef flat. This sump, protected from the force of the ocean waves by the algal ridge, allows us to draw water from beyond the reef crest. In certain weather conditions, water draining off the reef flat flows into the sump and delivers nitrogen-rich water to the microcosm (see Henderson *et al.*, 1976). On rare days during low spring tides when there is no surf action, the water in the sump is drawn down below the intake level so that the pumping ceases. A standpipe arrangement prevents the microcosms from draining, and the pump is re-started manually. The double pipe across the reef flat to the sump was emplaced during a quiet-water spring low tide and then secured with cloth bags which were filled, in place, with concrete and further anchored with reenforcing rods. This emplacement has now survived two seasons of winter surf. At the NUC facility a large amount of sand is

suspended in the surf zone and pumped into the facility. This problem is alleviated by delivering the water to a 2,500-liter settling tank which allows the sand to settle out. That sand is then cleaned out as needed.

C. Microcosm Aquaria, Associated Devices for Environmental Modification of Aquarium Characteristics

The microcosm tanks used in our facilities are constructed of 3/8" thick sprayed fiberglass. The inner dimensions are 117 cm by 117 cm by 46 cm deep. The total volume is 630 liters. A horizontal flange approximately 5 cm wide lends rigidity to the tanks. Tank dimensions should be kept constant for inter-tank comparisons. There are demonstrable microcosm community responses associated with surface to volume ratios and other "container effects." The water depth in the tanks is usually kept near 35 to 40 cm (480-550 liters). Water depth in the tanks and flow rate through the tanks are established by means of inlet and outlet head boxes. The water depth in the head boxes is fixed by means of adjustable standpipes (Figure A-2). Coarse control on the flow rate is controlled by the length of the standpipe. Screwing the standpipes up or down on threaded fittings provides fine control of flow. Such gravity control of flow rate is preferable to valves, which rapidly become fouled or clogged. With weekly cleaning of distribution lines, flow rates can be maintained to well within 5 percent of the desired rate by this system. The double standpipe arrangement also prevents the microcosms from draining in the event that seawater delivery stops. Flow rate through each microcosm is usually maintained near 10 liters/minute, so the flushing time of each aquarium is about 50 minutes. The drain on each outlet head box has a simple, automatic (inverted-U) siphon so that the head box fills, starts the siphon, drains, stops the siphon, and then repeats the cycle. Flow through the tanks is precisely measurable by timing the filling rate of the head boxes. Such a measurement system could be automated by installing electrodes which registered the time at two known points (hence, volumes) on the filling cycle. We have not found a preferable inexpensive flow meter which neither interferes with flow nor remains unaffected by fouling in the pipes.

Water flows into the aquaria via a pipe (actually, one of two; one for each of the duplicate distribution systems) near the center of the aquaria. The water wells up, mixes through the tank, and exits through another (again, one of two) pipe near one corner of the tank. An earlier configuration of the inlet induced a horizontal rotary flow from a point inlet on one side of each tank. This resulted in a conspicuous gradient in the fouling community around the tank sides. Such a gradient induced by variable water motion precluded convenient sampling of an homogenous community; the gradient has been eliminated by the present design.

Water composition can be varied by initial choice of water source (fresh water, bay water, well water), by manipulation before the water enters the inlet head boxes, by mixing in the head boxes or the inlet lines, or by direct additions to the tanks themselves. Descriptions of how we alter water composition follow.

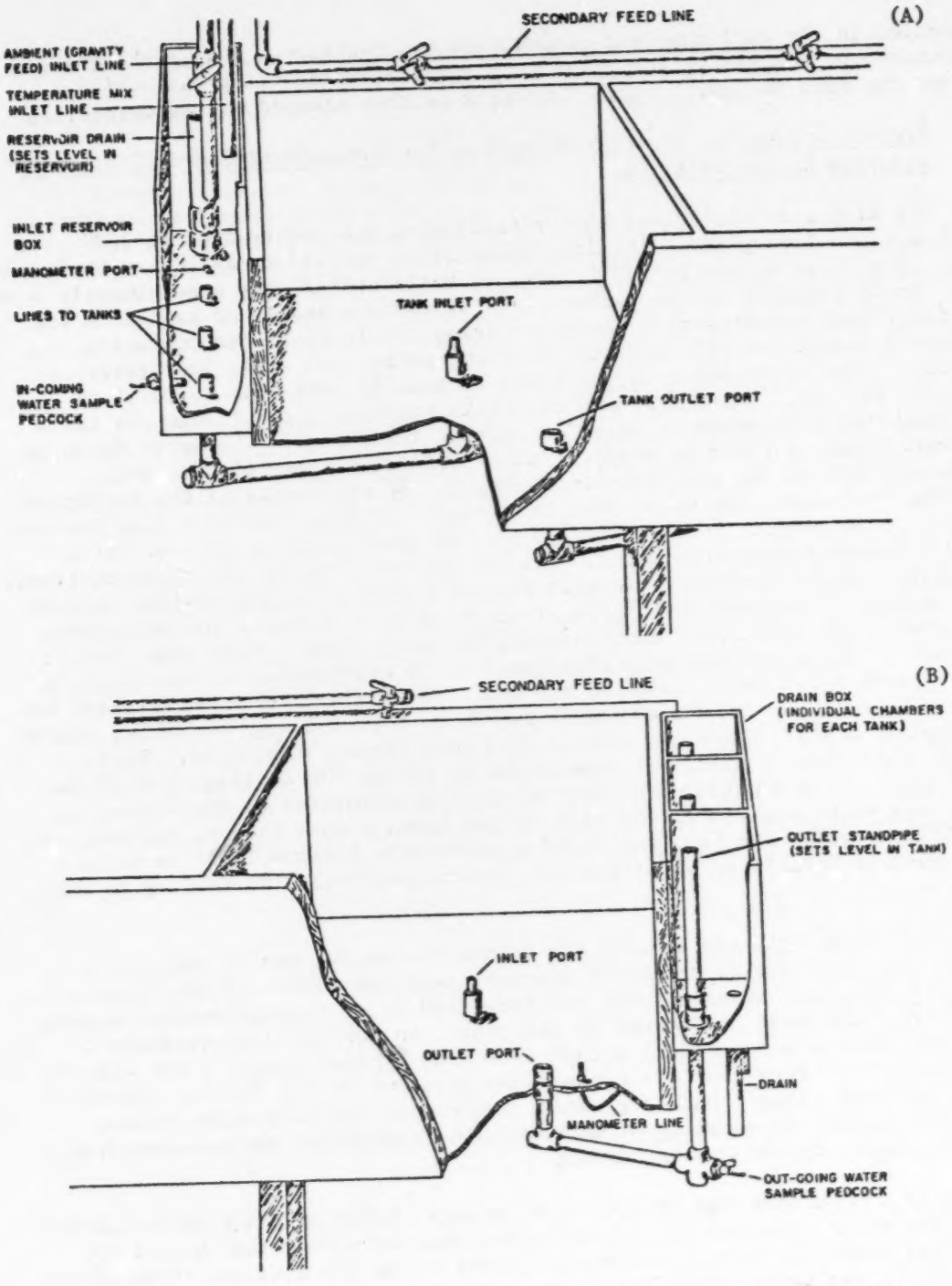

Figure A-2. Arrangement of standpipes in inlet (A) and outlet (B) head boxes.

Salinity is decreased by adding fresh water to the head box at a fixed rate. The fresh water is delivered by gravity feed from a large constant-head reservoir, rather than being supplied directly from the freshwater line which is subject to variable pressure and flow rate.

Dissolved inorganic materials (nutrients, toxins, etc.), are added to the inlet boxes or inlet lines by means of a peristaltic pump drawing from a stock solution in distilled water. By using a stock solution which is highly concentrated relative to the desired dissolved material concentration in the microcosm (e.g. 1,000-fold), one can avoid lowering the microcosm salinity with the additions. Cations can often be added as chloride compounds, while anions are often best added as sodium compounds. Because chloride and sodium are the two most abundant ions in seawater, use of such compounds minimizes the effects of added materials other than those of specific interest. Of course, salts of sodium or chloride are not always available or practical. Consideration of available salts and seawater chemistry can suggest the most viable choices. For nutrient additions under less precisely controlled conditions, we have also used high-nutrient water from a seawater well (see Table 8).

Temperature is controlled by heating or chilling the inlet stream with a heat exchanger system (Jokiel *et al.*, manuscript) or by placing glass resistance heaters in the head boxes. The former approach allows efficient and sophisticated control but involves extensive construction; the latter is simple to operate, but energy-intensive.

Dissolved oxygen concentration is lowered by bubbling nitrogen through the inlet head box. It is possible by this method to achieve a wide variety of oxygen levels simply by varying the nitrogen bubbling rate and monitoring the water until the desired oxygen level is obtained. If incoming water has low or variable oxygen level, the values can be brought to near saturation by cascading the water across ripple panels.

D. Automatic Sampling and Data Acquisition System

1. Automatic water sampling system--The purpose of this system is to control water flow from various sources to the measurement devices. The unit is under the control of a data logger which advances solenoid values from one water source to the next after each measurement sequence. Each channel is identified by a feedback signal to a data logger input channel.

Each water source being monitored is connected via a normally closed solenoid valve to a common manifold that directs water to the test electrodes. Each valve is actuated in order by the sequencer.

Various electrical, pneumatic, or hydraulic valves are available, and different sequence control mechanisms can be used to meet specific needs. Multiple port distribution valves operated by stepping motors can hydraulically or pneumatically trigger the water sampling valves in sequence. Electrically controlled valves are also available. New products are constantly coming onto the market but the following description of the HIMB prototype will serve as an example:

Water flow from the various sources is controlled by 20 remote-controlled normally-closed one-inch valves. The valves we have used at HIMB are Toro Company Model 202-08-04 units; these are made of plastic and offer no resistance to flow when open. The valves were modified by replacing the hard rubber seating gasket with soft 1/4 inch neoprene material to improve seating. These valves are hydraulic, operating off freshwater line pressure; they are actuated by 24 volt current to small Automatic Switch Company Model 8320A136 control valves. The fresh water is isolated from the seawater stream.

The configuration of the HIMB water sampling system is shown in Figure A-3. Only one solenoid is activated at any given time by an electrical signal from the data acquisition system. Water flushes the system for one minute before data from the probes are recorded. Specific flushing time should consider maximum volume of the lines through which the water flows, minimum flow rate of the water, and maximum response time of any in-line probes. The manifold is designed to insure complete flushing; stagnant areas are eliminated by use of a loop. Differences in flow over the probes that occur due to variation in length of hose can influence readings in the oxygen electrode. Constant flow over the probes at any water source pressure is accomplished by using a double standpipe arrangement (Figure A-4). Excess water spills over the first standpipe; a constant difference in height and thus constant flow over the probes is maintained.

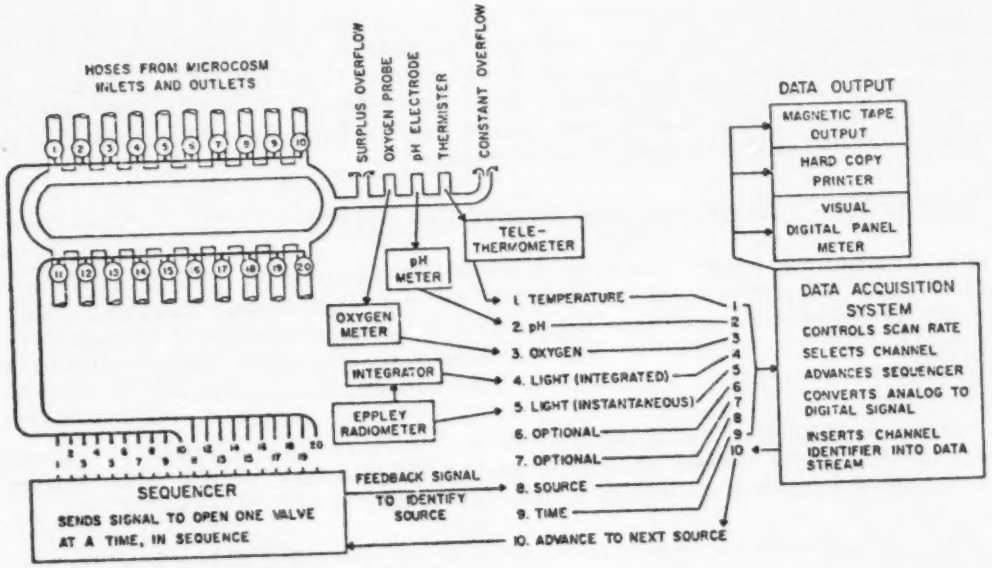

Figure A-3. Schematic diagram depicting the HIMB water sampling system.

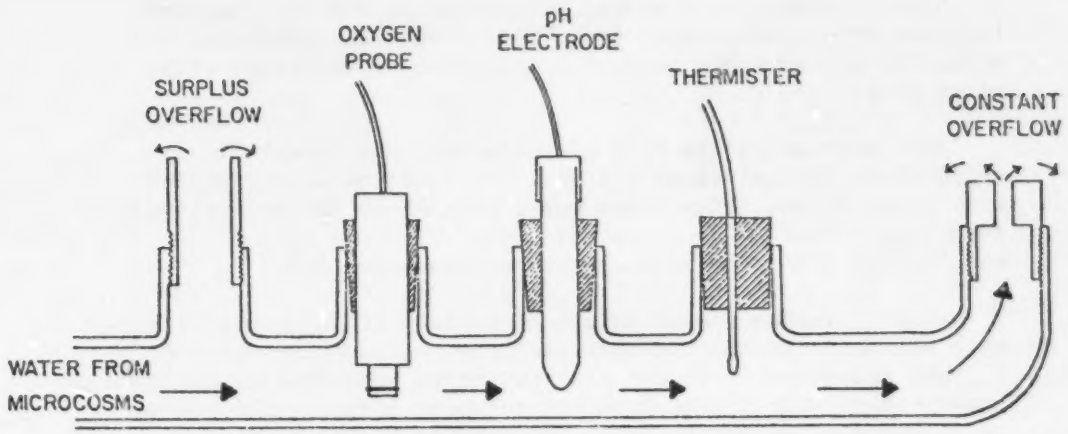

Figure A-4. Enlargement of area of water sampling system (see Figure A-3) which measures oxygen, salinity and temperature.

2. Automatic data acquisition system--Electronic data-loggers and electronic measurement probes can be used to monitor water composition and other variables in microcosms. Many components of the systems utilized in this program were designed and built locally by electronic technicians to meet specific needs; since then, versatile commercial data acquisition systems have become available. The data acquisition system is simple in concept. The acquisition system signals the water sampling system to shunt water from various sources past electronic sensors. Outputs from the sensors are recorded on magnetic tape and/or on a hard-copy printer. A properly designed system of this type is reliable and relatively easy to operate. The major problem usually centers on designing, building, and "debugging" the unit. The total system consists of measurement devices and a data logger system.

A variety of useful measurement devices are available. The system utilized in this program normally measures the following: light, temperature, dissolved oxygen, and pH. Additional channels are available for input from other instruments (turbidometer, fluorometer, specific ion electrode, conductivity meter, etc.), as required. Certain electrodes can interfere with one another through the common ground of seawater (e.g. oxygen and pH electrodes), so care must be exercised in matching instrument types. Examples of instruments used successfully with this system are as follows:

Temperature: An electronic telethermometer (Yellow Springs Instruments Model 47) with epoxy coated probe (Model 402) was utilized; numerous other products would be equally suitable. The probe is replaced with a 5 k-ohm variable resistor when intercalibrating the instrument with the data acquisition system.

Oxygen: Units with stable battery power sources that are isolated from line voltage (e.g. Yellow Springs Instrument Model 57) are unlikely to lose calibration or interfere with other sensors.

pH: Most pH meters have circuits designed to measure ungrounded test solutions and will not function in a grounded salt water stream. The Orion model 801A meter has an isolated test loop. This meter is satisfactory when used with a combination electrode such as the Beckman Model 3900.

Light: Instantaneous values of ambient light are measured by a standard radiometric cell and/or are accumulated value from a light integrator. In the past few years commercially produced quantameters with integrators and recorder outputs have become available (Lambda Instruments) and provide a measure that is superior to radiometric values for biological studies.

Our test instruments are obsolete, because they produce analog outputs which must be converted to digital values in the data logger. Analog signals are prone to transmission interference. A superior system would utilize digital output measurement devices exclusively. Converters could be installed directly on analog output instruments that could not be replaced with digital output units. We have been able to eliminate interference with our analog signals by shielding the wires and removing sources of electromagnetic noise (high voltage transformers, large motors, etc.), from the immediate vicinity of the data acquisition system.

Up to 12 microcosms are usually monitored simultaneously in our system, although fewer units are used for some experiments. The data acquisition system is usually set to scan at one-minute intervals, but six different scanning speeds are available on our logger. Eighteen "water sources" (twelve microcosm outlets, four inlet head boxes, water from an aerated saturation bath for O_2 probe calibration, and one source in which the O_2 meter is grounded to zero as an event mark) are monitored in sequence. On each scan, the following variables are recorded or cassette magnetic tape: water temperature, dissolved oxygen, pH, integrated total light, "mark" (a feedback voltage from the water sampling system used to identify each water source) and "time" from an internal digital clock. The data logger identifies each of the eight data channels on the tape by inserting a channel mark. Use of the YSI Model 57 oxygen meter has eliminated calibration drift encountered with the older YSI Model 54, and use of the aerated calibration water source on each cycle probably could be discontinued. That calibration bath still serves as an excellent check and reference mark in the data stream.

Data cassettes are transcribed onto 9-track tape for use on an IBM 370 computer system. The data are formatted into rows and columns and printed for editing before data analysis.